Indium-111-Labeled Platelets in Monitoring Human Pancreatic Transplants

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We have performed 59 ^{111}In-labeled platelet scintigraphies in 12 patients with pancreas transplant, and we have compared retrospectively the ^{111}In platelet uptake with the graft immunological situation. A diffuse uptake in the graft was seen in five of six patients with pancreatic rejection. The scans became positive before changes in biochemical tests were detected. No ^{111}In platelet uptake was seen in five of seven normally functioning grafts. Two cases of venous thrombosis and two perigraft hematomas appeared like a focal ^{111}In platelet accumulation. Indium-111-labeled platelet scintigraphy can be a useful method for monitoring pancreas transplants. It may be helpful in the early detection of pancreatic allograft rejection and in the differential diagnosis between this and other complications such as thrombosis or hematomas.

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The main clinical problem in pancreas transplant (PT) management is pancreatic allograft rejection (PAR), a common cause of graft failure (*1*). Early detection of PAR is of critical importance to start successful treatment.

Actually, the most employed techniques for the PT monitoring do not allow to detect the PAR early enough to make the antirejection therapy effective. Hyperglycemia requires destruction of more than 90% of the pancreatic B-cells (*2*). C-Peptide levels are a more sensitive indicator of PAR, but they are rarely available in time to be of clinical value. In patients with exocrine pancreatic secretions drained into the urinary system, urinary amylase levels are more sensitive than hyperglycemia in detecting pancreatic allograft failure (*3,4*).

Ultrasound, computed tomography and nuclear magnetic resonance images are helpful in the detection of structural abnormalities and fluid collections which appear as postoperative complications or in a later phase of PAR (*5–8*).

The earliest phase of PAR consists of interstitial cellular infiltrates and thrombotic vasculitis (*9,10*). This fact suggests that indium-111 (^{111}In) platelets can be a good tracer for the early detection of PAR, as has been demonstrated in renal transplantation (*11,12*). Based on the good results obtained with ^{111}In-labeled platelet scintigraphy (In-PS) in the early detection of renal graft rejection (*11,12*), we have used this technique for monitoring PT, and we report our experience on this topic.

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PATIENTS AND METHODS

Since February 1983, five isolated PT in diabetic patients and 15 simultaneous renal-pancreatic transplants in diabetic patients with end-stage renal failure have been performed in the transplant unit of our hospital.

We have studied 12 of these 20 patients, having performed 17 platelet labelings and 59 In-PS (Table 1). All patients were under immunosupressive medication: six with Azathioprine and Prednisone and the other six with Cyclosporine A.

In the first eight labelings, autologous platelets were labeled with [^{111}In]oxine by Thakur's method (*13*). In the remaining nine [^{111}In]mercaptopiridine was used for platelet labeling (*14*), as follows: 49 ml of venous blood were obtained, 34 ml over 6 ml of anticoagulant A (25.0 mg of 2-hydrated trisodium citrate and 14.9 mg of monohydrated citric acid per ml of solution) and 15 ml over 1.5 ml of anticoagulant B (3.8% sodium citrate). Cell separation was carried out as described by Thakur et al. (*13*). Thereafter, the platelet button was resuspended in 0.5 ml of platelet-poor plasma (PPP-A) and 2.5 μg of Merc in 10 ml of an aqueous solution was added. The tube was incubated for 10 min at room temperature. Subsequently 200–400 μCi of ^{111}In (75 μl of $^{111}InCl_3$ dissolved in 0.04 N HCl and 25 μl of citrate buffer 1*M* pH 6.5) was added to the cell suspension and the mixture was incubated

TABLE 1
Patients Studied by ^{111}In-Platelet Scintigraphy

Patient no.	Age (yr)	Sex	Transplant*	Pancreatic graft	Localization	Label no.	Number of In-PS
1	34	M	R + P	Segmentary	LIR	1	2
2	28	M	R + P	Segmentary	LRR	1	4
3	59	M	R + P	Segmentary	LRR	1	3
4	28	F	R + P	Segmentary	LRR	1	2
5	22	F	R + P	Total	RIR	1	2
6	32	M	P	Total	RIR	3	11
7	32	M	R + P	Total	RIR	2	10
8	35	M	R + P	Total	RIR	2	7
9	37	M	R + P	Total	RIR	1	4
10	34	M	P	Total	LIR	1	1
11	42	M	P	Total	RIR	2	9
12	43	M	R + P	Total	LIR	1	4

M = male, F = female, R + P = renal and pancreatic, P = pancreatic, LIR = left iliac region, LRR = left renal region, RIR = right iliac region.

* All were cadaver donors transplants. Exocrine secretions were drained into the urinary system except in Patient 1, in whom a duct occlusion by injection of a synthetic polymer was performed.

for 20 min at 37°C. The tube was centrifuged 10 min at 1000 *g* to eliminate the unbound indium activity and the platelet button was washed with PPP-A and subsequently resuspended in 4 ml of PPP-B. Finally, we reinjected 100–200 μCi (37-74 MBq) of ^{111}In platelets.

Scintigraphic images were obtained every day beginning at 24 hr postinjection (from 1 to 7 days, mean 3.47 days). Three patients required two platelet labelings and one Patient 3 (Table 1). Scintigraphic images were obtained with a Dyna 4/15 Picker gammacamera, using a medium-energy, parallel hole collimator and taking the two energetic peaks of ^{111}In (247 and 173 keV). The gamma camera was connected online with a PDP11 Digital computer, and 15-min digital images were recorded for quantitative analysis. Analogic images were evaluated qualitatively. Quantitative analysis was made in 30 scans by calculating the allograft/adjacent tissues (A/AT) ratios (Fig. 1).

Situations of PAR or immunologic tolerance were retrospectively established by the evaluation of clinical data (fever, abdominal pain) and complementary tests (glycemia, urinary amylases, C-peptide, ultrasonography) available during the In-PS study for each patient, and, in many cases, by the graft evolution.

RESULTS

We have observed three different patterns in the qualitative evaluation of the analogic images: (a) diffuse accumulation of ^{111}In platelets in the graft area, (b) focal accumulation of ^{111}In platelets either in the graft area or in adjacent areas, (c) no ^{111}In-platelet accumulation.

Results are summarized in Table 2. No ^{111}In platelet accumulation was observed in 23 scans (five patients, six platelet labelings) (Fig. 2). A "diffuse" pattern was seen in 21 scans (six patients, six labelings) (Fig. 3), and a "focal" pattern in 11 scans (four patients, four label-

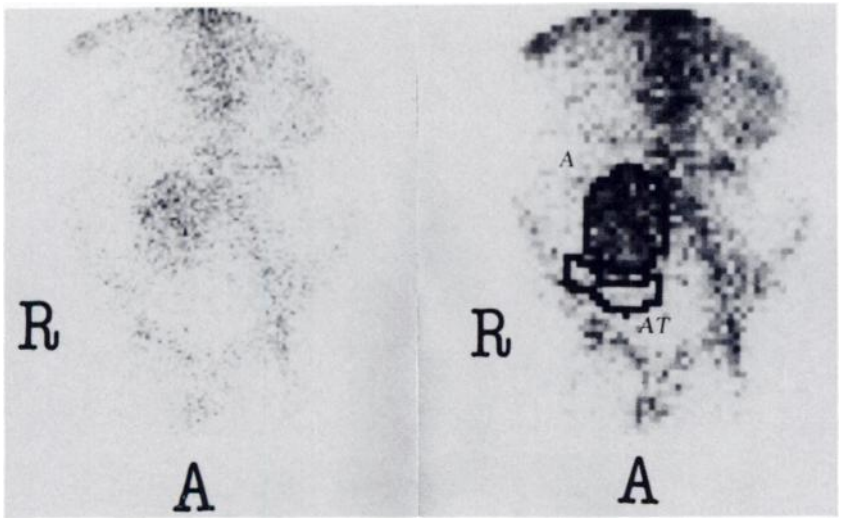

FIGURE 1
Allograft/adjacent tissues ratio. AnROI within the graft area or the ^{111}In-platelet accumulation (A), and a semicircular area including activity from adjacent tissues and iliac vessels (AT). Patient 9. Diffuse ^{111}In-platelet uptake corresponding to a PAR. A/AT = 1.62.

TABLE 2
Scintigraphic Pattern, Biochemical Tests, and Final Diagnosis Relationship

Patient no.	Labeling no.	Scintigraphic pattern	↑Glycemia	↓Urine amylase	↓C-peptide	RAR	Final diagnosis
1	first	Diffuse	No	No	No	Yes	NFP-RAR
2	first	Negative	No	No	No	Yes	NFP-RAR
3	first	Focal	No	Yes (1 day*)	Yes	No	VT
4	first	Focal	No	No	No	No	PH
5	first	Negative	No	No	No	Yes	NFP-RAR
6	first	Diffuse	Yes (3 days)	Yes (3 days)	—	No	PAR
6	second	Negative	No	No	No	—	NFP
6	third	Foc.-diff.	Yes (4 days)	Yes (4 days)	Yes (4 days)	—	PAR
7	first	Negative	No	No	No	No	NFP
7	second	Negative	No	No	No	No	NFP
8	first	Diffuse	No	No	No	Yes	NFP-RAR
8	second	Negative	Yes (5 days)	Yes (6 days)	Yes (5 days)	No	PAR
9	first	Diffuse	Yes (4 days)	Yes (3 days)	—	Yes	PAR
10	first	Focal	No	No	No	—	VT
11	first	Focal	No	No	No	—	PH
11	second	Diffuse	No	Yes (5 days)	—	—	PAR
12	first	Diffuse	No	Yes (1 day)	No	No	PAR

RAR = renal acute rejection; NFP = normally functioning pancreas; VT = venous thrombosis; PH = perigraft hematoma; PAR = pancreas allograft rejection.
* Days after the first In-PS.

ings) (Fig. 4). Patient 6 had a focal accumulation in the first two scans (third labeling), which became diffuse in the later ones.

Four of the six patients with diffuse ^{111}In-platelet uptake were in a situation of PAR with fever. Hyperglycemia and decreased urinary amylase levels appeared several days after the first scan (Table 2). One of these four patients had renal graft rejection which also showed platelet uptake (Patient 9). We have not been able to establish the PAR diagnosis in the other two patients (1 and 8). Both of them had acute renal graft rejection but normally functioning pancreatic grafts with good evolution (Fig. 5).

A focal ^{111}In platelet accumulation was found in four patients (Table 2). The diagnosis of venous thrombosis

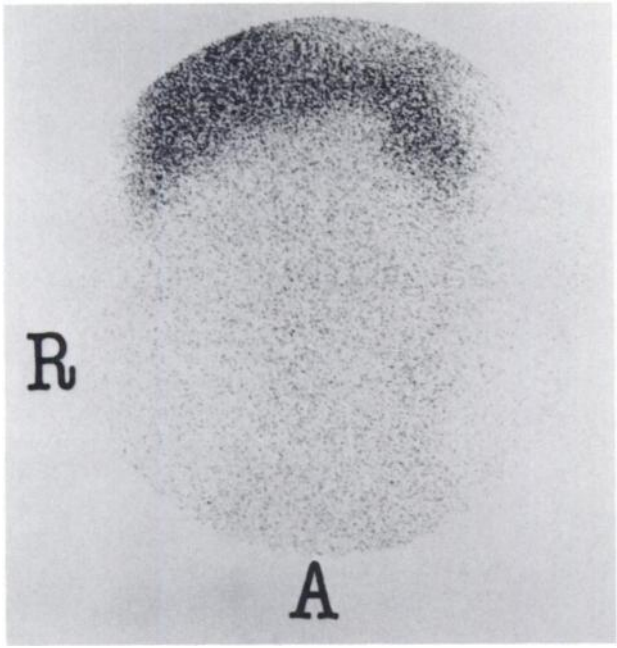

FIGURE 2
Negative In-PS. Patient 5. Good pancreatic function.

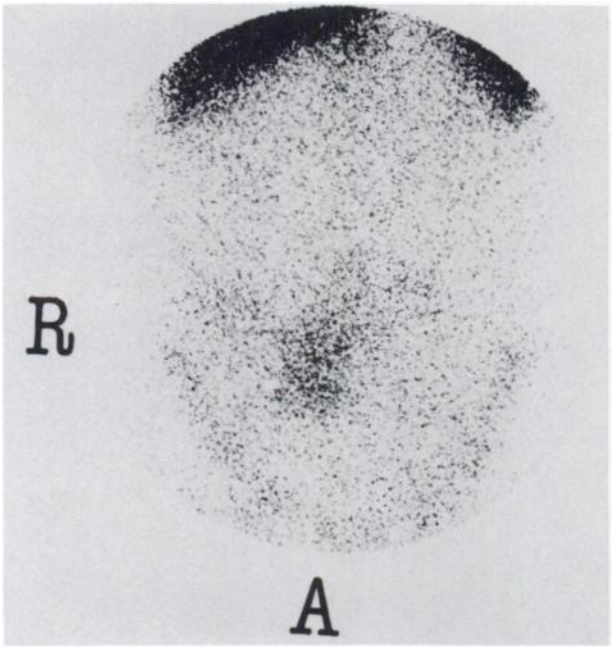

FIGURE 3
Diffuse ^{111}In-platelet uptake over the graft area (Patient 6, study 1). A/AT = 1.65. A PAR was diagnosed.

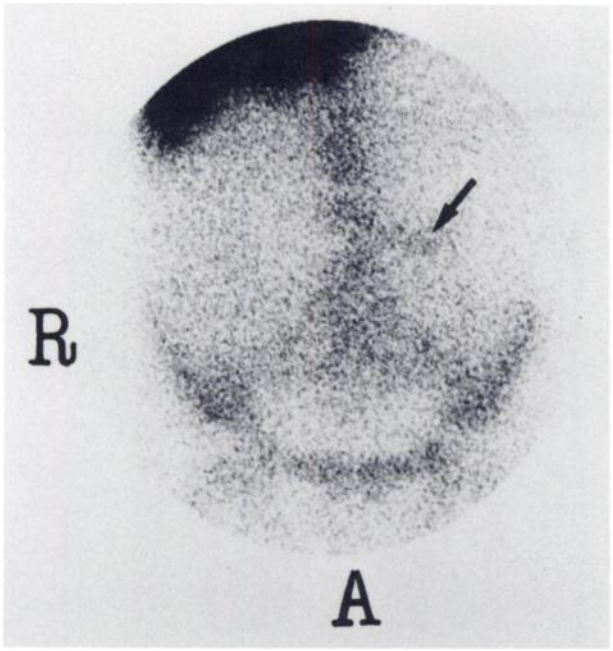

FIGURE 4
Focal ^{111}In-platelet uptake (arrow), corresponding to a venous thrombosis (Patient 3).

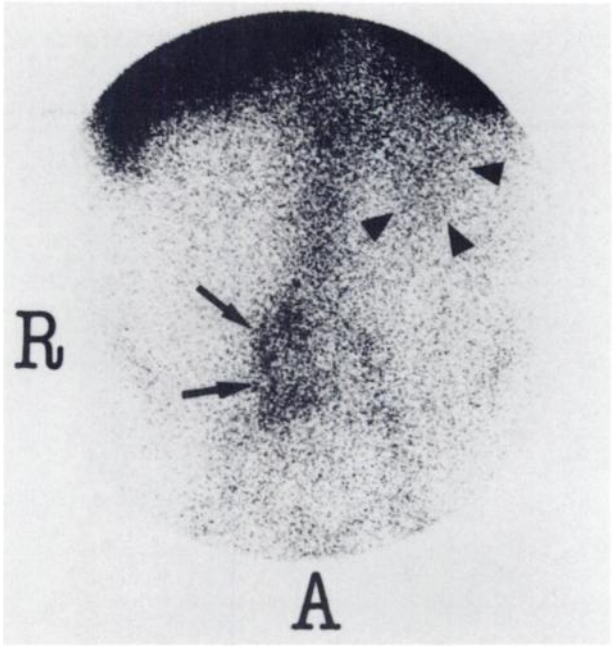

FIGURE 5
Diffuse ^{111}In-platelet uptake in a normally functioning pancreatic graft (arrows). Acute renal graft rejection, with ^{111}In-platelet accumulation (arrow heads) (Patient 8).

was achieved in two cases, confirmed in one case at transplantectomy and in the other one by ultrasound-Doppler and angiography. A perigraft hematoma was suspected in the remaining two cases, which was confirmed in one patient by needle aspiration. In spite of a strong clinical suspicion of perigraft hematoma in the other patient (important postoperative bleeding), it was not possible to demonstrate it.

The recipient who had a focal pattern in the first two scans which became diffuse in the later images, presented hyperglycemia and a fall in the C-peptide and urinary amylase levels 4 days after the first scintigraphic image, confirming PAR (Patient 6).

No ^{111}In-platelet accumulation was observed in five patients, in which six platelet labelings were performed (Table 2). Four patients (five labelings) had good pancreatic function. Two of them were under antirejection therapy for acute renal allograft rejection. In the remaining patient (8) PAR was diagnosed by biochemical tests that appeared abnormal 5 to 6 days after the first In-PS.

The results of the A/AT indexes calculated are summarized in Table 3. We have not found significant differences in the A/AT ratio between "diffuse" and "focal" positive scans.

DISCUSSION

PAR diagnosis is a main problem in the management of patients with PT. As explained before, biochemical tests and especially urinary amylases are frequently used for the diagnosis of PAR. However, in our experience In-PS has shown platelet accumulation before a decrease in urinary amylase levels was detected (see Table 2).

Scintigraphic methods for the evaluation of PT have been reported. The uptake of selenium-75 selenomethionine by the graft has been suggested as an alternative, but a decrease in the uptake coincides with the increase in serum glucose levels, and therefore it is not a useful method for the early detection of PAR (*15*). Vascular tracers have allowed the evaluation of the allograft perfusion. Shulkin et al. (*16*) used technetium-99m diethylenetriaminepentaacetic acid (DTPA) in seven recipients of renal and pancreatic allografts, finding a decreased perfusion in a PAR before elevation of the blood glucose occurred. George et al. have evaluated the pancreatic allograft perfusion in a review of 209 [^{99m}Tc]sulfur colloid and [^{99m}Tc]glucoheptanate scintigrams. They calculated a "thrombotic index" and they found different patterns for acute rejection, pancreatic infarction, pancreatitis and atrophy (*17*). In our experience, the interpretation of these vascular images of the pancreatic grafts is difficult, because most of them are implanted in the lower abdomen, so activity from iliac vessels and splanchnic pool is superimposed. Moreover, in spite of the prognostic value of these scintigraphic perfusion studies (an absence of perfusion leads in most cases to graft rejection), they are not specific, and cannot differentiate between PAR and thrombosis in the vascular anastomosis.

There is little experience with ^{111}In-platelet scintig-

TABLE 3
Scintigraphic Pattern and A/AT Ratio Relationship

Patient no.	Labeling no.	Scintigraphic pattern	A/AT	Date	$\bar{x} \pm \delta$
6	1	Diffuse	1.35	04/30/85	1.58 ± 0.20
			1.65	05/02/85	
			1.49	05/03/85	
8	1	Diffuse	1.75	11/19/86	
			1.63	11/22/86	
9	1	Diffuse	1.43	11/20/86	
			1.62	11/21/86	
			1.98	11/22/86	
11	2	Diffuse	1.77	09/23/87	
			1.36	09/25/87	
			1.36	09/26/87	
10	1	Focal	1.46	03/18/87	1.53 ± 0.14
			1.47	03/20/87	
			1.40	03/21/87	
11	1	Focal	1.35	06/11/87	
			1.57	06/12/87	
			1.76	06/13/87	
			1.68	06/15/87	
7	1	Negative	1.03	03/14/86	1.06 ± 0.09
			1.09	03/15/86	
			1.01	03/17/86	
			1.02	03/19/86	
			0.98	03/20/86	
			1.03	03/21/86	
			1.11	03/22/86	
7	2	Negative	0.99	05/08/86	
			1.01	05/09/86	
			0.98	05/10/86	
8	2	Negative	1.22	12/18/86	
			1.24	12/19/86	

raphy in pancreatic transplant. Sollinger et al. reported the value of this technique in the early detection of PAR and its effectiveness in the evaluation of the rejection therapy in dogs (*18*). Jurewicz et al. have studied with In-PS 11 recipients of renal and pancreatic allografts making quantitative and qualitative analysis of the graft tracer uptake. They have not found platelet accumulation in the normally functioning grafts, and concluded that In-PS was not only helpful in the early diagnosis of graft failure, but also in recognizing other complications such as thrombosis or perigraft hematoma (*19,20*).

In our series of patients, we could recognize two venous thromboses and two hematomas. In these cases we found a focal ^{111}In-platelet accumulation. When this pattern is seen out of the graft area, it is easy to diagnose a perigraft hematoma, but the interpretation can be difficult when this image is extensive or it is within the graft area.

Situations of PAR corresponded to a diffuse ^{111}In-platelet accumulation except in one case, in which the scans were negative. Prophylactic administration of immunosupressive agents can be a cause for false-negative images. A diffuse pattern was also observed in two patients with normally functioning grafts. These patients were under antirejection therapy for acute rejection of their renal graft. In patients with simultaneous renal and pancreatic transplants, antirejection therapy is often started if renal allograft rejection is suspected. In these patients, it is possible that the pancreatic graft was in an early stage of rejection that could permit the platelet accumulation. It is possible though, that the pancreatic function was reestablished because of these therapeutics before changes in biochemical tests appeared. If that was the case, we couldn't consider the ^{111}In-platelet uptake as a false-positive result.

We have little experience in the quantitative analysis of ^{111}In-platelet uptake in PT. In isolated renal transplants, we obtain a quantitative approach by calculating the renal graft/contralateral area index (*11,12*). However, this is not applicable in PT, specially in those patients who have a renal graft in the contralateral area. For this reason we have tried when calculating the index a peripancreatic area of reference, which has to include iliac vessels activity if the pancreas graft is over these vessels. We have found differences in the mean value

of the A/AT ratio between the positive and negative scans, but not between focal and diffuse patterns. This is in agreement with the results of Jurewicz et al. We believe that further experience is needed in order to establish numerical limits.

CONCLUSION

We agree with other authors that In-PS can detect the PAR in its earlier stage, and that it may be helpful in the differentiation between PAR and other PT complications, like thrombosis or hematomas. Although further experience is necessary before definitive conclusions can be made, we believe that ^{111}In-platelet scintigraphy may be a useful noninvasive method for PT monitoring.

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