Imaging of Cardiac Transplantation Rejection in Primates Using Two New Antimyosin Agents

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Indium-111-labeled monoclonal antimyosin Fab has been used to image myocardial infarction, myocarditis and cardiac transplant rejection with localization in myocytes that have suffered irreversible loss of cell membrane integrity. Technical factors potentially limiting clinical usefulness of ^{111}In antimyosin include dosimetry (72 hr half-life of ^{111}In), slow blood clearance of antibody proteins delaying optimal imaging to 24 to 48 hr postinjection and nontarget organ uptake. Therefore, two new antimyosin imaging agents experimentally shown to potentially improve dosimetry, shorten time from injection to imaging or decrease nonspecific cell binding were evaluated in a primate cardiac transplant model. The two agents evaluated were polylysine ^{111}In-antimyosin (0.023 mg Fab modified with a 3.3 kd polymer of polylysine and labeled with ^{111}In) and ^{99m}Tc-antimyosin (0.5 mg Fab' antimyosin labeled using the RP-1 ligand technique). A total of eight baboons were studied: three with heterotopic (cervical) xenographs, three with orthotopic allographs and two control animals. Each animal was injected first with 12-23 mCi of ^{99m}Tc-RP-1 antimyosin and 5-16 hr after completion of imaging, was injected with 0.72-1.88 mCi of ^{111}In-polylysine antimyosin (PIs) and reimaged 12-48 hr later. The imaging results were compared to the histology of the animals. Biexponential curves were fit to the blood sample data and rate constants were determined and expressed as $T_{½}$ values. There were no significant differences between the two agents in either the early fast components or the late slow components. On planar imaging, there was blood-pool activity at 10-12 hr postinjection of both agents, but by 16-24 hr postinjection, blood pool was negligible on the ^{111}In-PIs scans. Both agents were concentrated in the rejected cardiac tissue. The slow blood-pool clearance combined with the 6 hr half-life of ^{99m}Tc-RP-1 AMA make this agent less promising for detection of diffuse myocardial uptake than ^{111}In Fab modified with polylysine.

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The current method used to diagnose allograft rejection is the endomyocardial biopsy and the hallmark of rejection is monocellular infiltration and myocyte necrosis (*1*). The endomyocardial biopsy is invasive, costly and time-consuming. A potential noninvasive method to diagnose cardiac transplant rejection is scintigraphic imaging of myocardial uptake of radiolabeled monoclonal antimyosin Fab fragments. Myocyte necrosis is a hallmark of cardiac allograft rejection. Antimyosin localizes in myocardial cells that have suffered irreversible ischemic injury with loss of cell membrane integrity (*2,3*). Experimental studies have shown myocardial uptake of antimyosin in animal models of myocarditis and cardiac transplant rejection (*4,5*). Clinical studies have documented the sensitivity and specificity of antimyosin imaging for detecting myocarditis and cardiac allograft rejection (*6–11*). The antimyosin product in clinical use is a murine monoclonal Fab fragment directed against intracellular heavy chain human myosin that is linked to a bifunctional chelating agent diethylene triamine pentaacetic acid (DTPA) and radiolabeled to ^{111}In.

Despite well established clinical uses there are three technical limitations to ^{111}In-antimyosin. The long (72 hr) half-life of ^{111}In limits the administered dose to 2 mCi and the number of possible injections per year. The dosimetry is a particular drawback if multiple studies are needed for surveillance in cardiac transplant patients. The second limitation is slow blood-pool clearance of a protein bound imaging agent. The predominant late component of blood clearance of ^{111}In-antimyosin has a half-life of 12 hr (*12*). To ensure blood-pool clearance, optimal clinical imaging is delayed 24–48 hr postinjection. This is a drawback for the use of this technique to noninvasively diagnose rejection in an outpatient post-cardiac transplantation setting. The third limitation is hepatic sequestration of chelated radiolabeled antibodies and high activity in nontarget organs adjacent to the heart which may interfere with the visualization of cardiac uptake of antimyosin.

A method to label Fab antimyosin with ^{99m}Tc has been developed (*13*). Technetium-99m is readily available (generator-produced) and when compared with ^{111}In has better imaging properties and improved dosimetry. Another approach to overcome some of the limitations of ^{111}In-Fab antimyosin has been to modify the ^{111}In-labeled antibody with negatively charged protein polymers to hasten blood clearance and increase target specific activity (*14–16*).

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This study evaluates and compares in a primate cardiac transplant model two new antimyosin Fab imaging agents: ^{111}In-Fab monoclonal antibody modified with a negatively charged synthetic low molecular weight polymer (polylysine) and ^{99m}Tc-antimyosin Fab' fragment modified with an ester RP-1 ligand. The goal of this study was to see if either or both of these agents offer potentially achievable improvements in time to imaging, patient dosimetry and/or nontarget-organ uptake over these same known properties for ^{111}In-antimyosin.

MATERIALS AND METHODS

Eight adult primates were used in this study with mean weight of 14.83 kg (range 4.1–23 kg). Three primates received cervical heterotopic xenographic heart transplants from cynomologus (macacus fasciularis) monkeys. Cardiac transplantation was performed by the method previously described by Jamieson et al. (*17*). Three primates underwent orthotopic allographic heart transplantation. Cardiac transplantation was performed by the standard orthotopic allographic technique. Two normal primates were also injected and one was imaged at corresponding times. Five of the six primates that had undergone cardiac transplantation received immunosuppressive therapy consisting of cyclosporine 15 mg/kg and depomedrol 0.8 mg/kg. Each primate received the same regime. Immunotherapy was stopped approximately 4-5 days prior to radiotracer injection. One transplanted primate did not receive any immunotherapy.

The time interval between surgery and radiotracer injection and imaging was greater than 6 mo in five of six primates receiving xenografts. One animal (#6) received an orthotopic graft, did not receive immunosuppressive therapy, then developed acute rejection 2 wk postoperatively and was then injected and imaged.

Preparation of the Antimyosin Antibodies

Polylysine antimyosin was supplied by Massachusetts General Hospital and prepared by the method described by Khaw et al. (*16*). The chelator DTPA was covalently linked to cationic synthetic polymer polylysine of molecular weight 3.3 kilodaltons by the mixed anhydride method described by Krajcarek et al., creating a DTPA-polylysine complex (*18*). The residual free epsilon amino acid groups of the DTPA-Pl complex were then succinylated (Pls) creating a negatively charged DTPA-Pls complex. This complex was then covalently bound to antimyoslin Fab fragments through a modified carbodiimide method (*14*). The DTPA-Pls antimyosin complex was then radiolabeled with ^{111}In via transchelation using 1 *M* of sodium citrate as a weak chelator. The ^{111}In-DTPA-Pls antimyosin complex was allowed to stand at room temperature for 15 min. Bound and free ^{111}In were separated on a Sephadex G-25 column. The peak tubes containing ^{111}In-DTPA-Pls antimyosin were pooled and radiochemical purity checked. With this method approximately 1 mCi of ^{111}In was labeled to 23 μg of DTPA-polylysine antimyosin.

Preparation of the ^{99m}Tc radiolabeled Fab' antimyosin was performed using an ester linked chelator conjugate RP-1 bound to Fab' prepared as previously described by Weber et al. (*13*) and supplied by Centocor (Malvern, PA). Briefly, the bifunctional ester conjugate RP-1 is attached to the sulfhydryl group of the C terminal region of the Fab' fragment by a method that preserves antigen binding sites. The bifunctional ester linked chelator RP-1 Fab' complex is then reconstituted with 2 ml of [^{99m}Tc] pertechnetate and allowed to stand at room temperature for 15 min. This allows the N3S core of the complex to bind to the negatively charged ^{99m}Tc. Radiopurity was checked using ITLC and for each preparation was greater than 90%.

Experimental Protocol

The primates were anesthetized, intubated with a cuffed endotracheal tube and ventilated with room air. Two peripheral intravenous cannulas were placed and used for administration of radiolabeled antimyosin and for blood sampling. Each primate was injected first with 12–23 mCi (mean 18.71 mCi) of ^{99m}Tc-RP-1, and after completion of imaging the primates were injected with 0.72–1.88 mCi (mean 0.98 mCi) of ^{111}In-DTPA-Pls antimyosin. Blood samples were obtained at 1, 10, 30, 60, 180, 540, 1440 and 2000 min postinjection to determine plasma clearance. One milliliter aliquots of each blood sample were counted at the same time in a Picker Spectroscaler 4R counter at a preset time of 5 min and a MeV range of 0.2. Upper and lower threshold settings for PHA were 450 and 950 for ^{99m}Tc-RP-1 and 250 and 900 for ^{111}In-Pls antimyosin. The 1-min sample was used as the 100% blood activity point and all blood samples were normalized to this point. Data from ^{99m}Tc-RP-1 and ^{111}In-Pls antimyosin disappearance curves were fit to a biexponential equation using a weighted least squares technique (*19*). All data points were combined into a single biexponential washout curve. From the rate constant of the washout curve, the half-life of the fast and slow components were determined.

Planar scintigraphic images were obtained at a mean of 8.5 hr from injection of the ^{99m}Tc-RP-1 and a mean of 24 hr for the ^{111}In-Pls antimyosin. These imaging times were selected for logistic reasons and because of the different half lives of the two radiotracers, however, several animals had sequential early and late imaging following injection of each agent. Five minute planar acquisitions were performed in the anterior and left lateral projection using a large field of view camera (Picker SX-300) equipped with a high-resolution, parallel-hole collimator and a 20% window centered on 140 keV photopeak of ^{99m}Tc and a medium-energy, parallel-hole collimator with 15% windows centered on the 169 and 240 keV photopeaks of ^{111}In. Early dynamic imaging following injection of ^{111}In-Pls antimyosin was performed in one animal to evaluate early tracer biodistribution.

To assess cardiac uptake of antimyosin from the planar scans a semiquantitative rejection score method was used (*7*). For the heterotopic transplant model a ratio of average counts per pixel for the graft over the native heart, each corrected for their respective background, was used. For the orthotopic transplant model average counts per pixel in the graft were divided by average counts per pixel in the lung. An antimyosin ratio greater than 1.6 was considered indicative of rejection (*7*). Visual interpretation and analysis was done by consensus among four observers blinded to the results of the histopathology. A progressive step score was used: no uptake, mild to faint uptake, moderate uptake, intense uptake (*7*). Blood-pool activity was considered present based on visualization of the great vessels.

Following completion of the imaging protocol, the rejecting heterotopic transplanted hearts were biopsied transcutaneously and representative tissue samples were analyzed. Twenty-four to 48 hr after imaging the heterotopic transplanted hearts were removed and orthotopic transplanted primates were killed. Representative tissue samples were obtained for histopathological examination. Rejection was graded semiquantitatively using the

modified Billingham method (*20*). The following grading scheme was followed:

0 no evidence of rejection
1 mild monocellular infiltration
2 moderate monocellular infiltration with rare myocyte necrosis
3 severe monocellular infiltration with sheets of myocyte necrosis.

The degree of rejection was graded from 0.5 to 3.0. Tissue samples were taken from the anterior and posterior wall of the RV and LV free walls and the intraventricular septum. Each free wall was divided into three zones corresponding to the epicardium, mesocardium, and endocardium. A final histological score was obtained by averaging the individual scores from all zones. Rejection scores derived from the antimyosin planar scans were correlated with either the biopsy results (heterotopic grafts) or the postmortem histopathology (orthotopic grafts).

RESULTS

Both radiolabeled antimyosin agents were administered intravenously without adverse effects in all eight primates. For ^{111}In-Pls antimyosin the mean Fab antimyosin dose injected was 22.7 μg with a mean ^{111}In dose of 0.98 mCi (range 0.34–1.88 mCi). The mean ^{99m}Tc-RP-1 dose was 18.7 mCi (range 12.2–23.5) for 500 μg of Fab′ antimyosin (Table 1). The standard ^{111}In dose used in clinical imaging is 2 mCi for an Fab dose of 500 μg (*12*).

The blood-pool clearance curves for ^{111}In-Pls antimyosin were biexponential and demonstrated a half-life for the initial fast component of 7.7 ± 12 min and a half-life for the predominant late slow component of 462 ± 204 min. Qualitative visual interpretation of antimyosin uptake in the ^{111}In-Pls antimyosin scans demonstrated no significant uptake in the normal primate, moderate uptake in four primates and minimal uptake in one primate (Table 2). Quantitative ^{111}In-Pls antimyosin uptake score was 1.9 ± 0.3 (range 1.5–2.28) for the primates that had undergone cardiac transplantation and 1.3 for the normal control primate. Residual blood-pool activity on scans varied according to imaging times. Two primates were imaged multiple times, one at 8.5 and 28.5 hr, and one at 10, 12 and 48 hr. At 8–10 hr, blood-pool activity was prominent, whereas at 28–36 hr postinjection, blood-pool activity was minimal or absent (Figs. 1, 2). The normal primate showed some residual blood-pool activity at 24 hr, which was gone at 36 hr postinjection. Two primates were imaged once at 24 hr postinjection and demonstrated negligible blood-pool activity. Biodistribution of ^{111}In Pls antimyosin demonstrated moderate hepatic uptake at 12–24 hr postinjection, but negligible uptake early (up to 4 hr).

TABLE 1
Results of ^{111}In and ^{99m}Tc Injections in Primates

Radioisotopes	Total Fab dose	Total dose in mCi
^{111}In-AM	500 μg	2.5 mCi
^{111}In-Pls AM	23.7 μg	0.98 mCi
^{99m}Tc-RP-1	500 μg	18.7 mCi

TABLE 2
Uptake Results After Injection and Imaging

Primate	Transplant	ITPI ^{111}In-Pls AM	ITPI ^{99m}Tc-RP-1 AM	^{111}In-Pis AM score	^{99m}Tc-RP-1 AM score	Biopsy score
1	Heterotope	8.5, 28.5	2.15, 5.3	1.5	2.4	1.45
2	Heterotope	24	8.4	1.8	1.84	2.66
3	Orthotope	24	10	2.28	2.28	1.55
4	Normal	24, 36	8	1.3	1.3	NB
5	Orthotope	10, 12, 48	16	1.75	2.6	1
6	Orthotope	12	9	NI	2, 6	1.6
7	Heterotope	NI	9	NI	2.6	2.5

ITPI = imaging time postinjection in hours; AM = antimyosin; NI = not imaged; NB = not biopsied.

The blood clearance curves for ^{99m}Tc-RP-1 were biexponential and demonstrated a half-life for the initial component of 8.3 ± 7.1 min and a half-life for the predominant late slow component of 462 ± 126 min. Quantitative ^{99m}Tc-RP-1 uptake scores in six of the seven primates imaged was 2.25 ± 0.15 (range 1.8–2.6). Qualitative visual interpretation of ^{99m}Tc-RP-1 uptake on planar scans demonstrated no significant uptake in the normal primate, moderate uptake in four primates and mild uptake in two primates. Significant residual blood-pool activity was noted in four primates at imaging times of 5.3, 8, 8.4 and 9.5 hr, respectively (Fig. 3). Blood-pool activity was minimal in two primates at 10 and 16 hr postinjection (Fig. 4). Biodistribution of ^{99m}Tc-RP-1 demonstrated minimal hepatic uptake in all seven of the primates imaged with uptake of the radiotracer in the kidneys and the bladder.

Orthotopic grafts imaged 10 and 24 hr postinjection showed good uptake of both tracers (Figs. 5 and 6).

Primate 4 did not undergo cardiac transplantation and had a low antimyosin score (1.3) for both imaging agents. There was no statistical difference between ^{99m}Tc RP-1 and ^{111}In-Pls antimyosin scores. The biopsy and postmortem histology correlation data are displayed in Table 2. Five of the six primates injected with ^{99m}Tc-RP-1 had score ratio >1.6 (mean 2.34 ± 0.31) associated with a mean

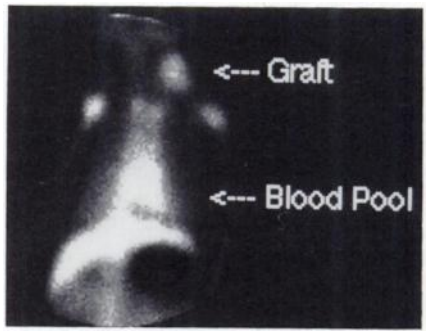

FIGURE 1. Anterior view of a primate with a heterotopic xenographic cervical heart transplant 8 hr after ^{111}In-Pls antimyosin injection. Scan demonstrates tracer uptake in the graft, liver and proximal humerus as well as prominent residual blood-pool activity.

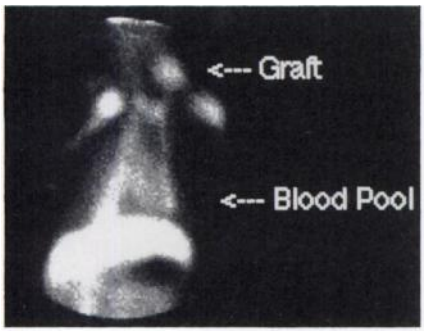

FIGURE 2. Anterior planar view of a primate with a heterotopic xenographic cervical heart transplant 24 hr after ^{111}In-Pls antimyosin injection. Scans demonstrate tracer uptake in the graft, liver and proximal humerus with minimal residual blood-pool activity.

Billingham biopsy score of 1.95 ± 0.58. Five primates injected with ^{111}In-Pls antimyosin had score ratios > 1.6, with a mean score of 1.83 ± 0.33 associated with a mean Billingham biopsy score of 1.82 ± 0.57. Primate 6 had a high score (2.2 and 2.6 for ^{111}In-Pls antimyosin and ^{99m}Tc-RP-1, respectively) associated with a low Billingham biopsy score showing minimal cellular infiltration but with prominent myocyte necrosis.

The heterotopic grafts were nonworking with minimal cavitary blood pool. It would be expected, therefore, that even for the early imaging time there would be a better correlation between counts and histopathology for the heterotopic xenographs than for the orthotopic allografts. The numbers were too small to make this comparison.

DISCUSSION

The available clinical method for surveillance and diagnosis of cardiac transport rejection is right ventricular endomyocardial biopsy. This procedure is invasive, expensive and may have low sensitivity due to sampling error. Antimyosin scintigraphy has demonstrated clinical utility in evaluating patients with myocarditis and cardiac transplant rejection (*6–11*). Modification in the preparation and radiolabeling of antimyosin antibodies has occurred to lower dosimetry, shorten blood-pool clearance and decrease nonspecific organ binding (*14,15,21,22*). In the present study of two new antimyosin imaging agents, ^{111}In Fab antibody modified with a negatively charged polymer polylysine and ^{99m}Tc-Fab′ RP-1 were studied in a primate cardiac transplant model.

Indium-111-Pls antimyosin is an antibody that has been modified by a negatively charged low molecular polylysine.

FIGURE 3. Anterior planar view of a primate with a heterotopic xenographic cervical transplant 5.3 hr after ^{99m}Tc-RP-1 injection. Scan demonstrates tracer uptake in the graft and kidney with significant residual blood-pool activity.

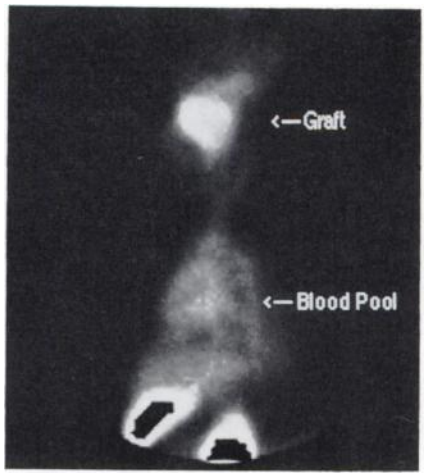

FIGURE 4. Anterior planar view of a primate with a heterotopic xenographic cervical transplant 10 hr after ^{99m}Tc-RP-1 injection. Scan demonstrates tracer uptake in the graft and kidneys with residual blood-pool activity.

The linking of polyvalent cations such as ^{111}In to protein polymers requires chelating agents to bind to the polymer. The higher the substitution of chelator groups on the polymer, the greater the number of moles of polyvalent cation binding to the polymer and the greater the specific activity of the radiolabeled antibody. However, increasing the number of chelator substitutions on the polymer can inactivate the antibody region of antigen-antibody recognition (*23*). Khaw et al. demonstrated that a chelator-to-polymer ratio of 4:1 was optimal for maintaining antigen-antibody binding capabilities and increases the ratio of moles of ^{111}In to moles of Fab to 10–15:1. The advantage of the increased ratio of moles of ^{111}In to Fab is to increase photon flux due to higher specific activity and decrease the total dose of antibody administered and decrease the administered dose of ^{111}In, all of which occurred in the present study.

By changing the antibody charge from positive to negative there is a decrease in nonspecific electrostatic interaction between positively charged antibodies and negatively charged cell membranes, as well as reduced nonspecific sequestration in nontarget organs such as the liver. In a canine model of myocardial infarction, Khaw et al. demonstrated that Pls antimyosin improved target to background activity, decreased hepatic uptake and decreased nontarget organ activity (*16*). Hepatic uptake was more

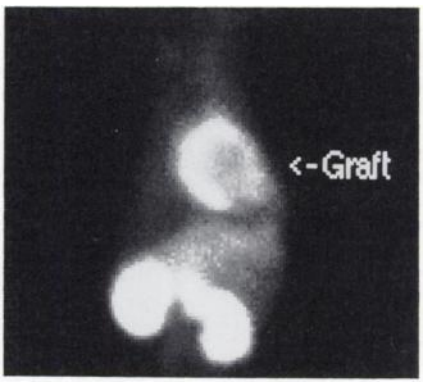

FIGURE 5. Anterior planar view of a primate with a orthotopic allographic heart transplant 9.5 hr after ^{99m}Tc-RP-1 antimyosin injection. Scan demonstrates tracer uptake in the graft and kidneys with some residual blood-pool activity.

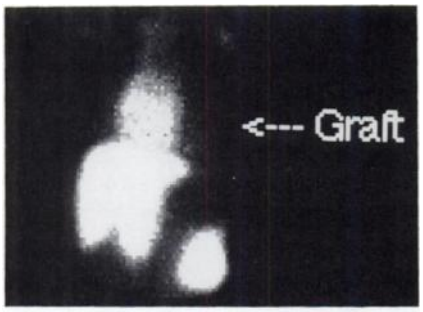

FIGURE 6. Anterior planar view of a primate with a orthotopic allographic heart transplant 24 hr after ^{111}In-Pls antimyosin injection. Scan demonstrates tracer uptake in the graft, liver and kidneys.

apparent in the primates imaged at 24 hr than canines imaged at 2–6 hr. The one primate imaged every 10 min up to 4 hr following ^{111}In-Pls antimyosin injection did not show hepatic uptake. Therefore, the late hepatic uptake in this model may be due to antibody alteration occurring with prolonged time in the circulation leading to uptake by the reticuloendothelial system in the liver.

An alternative method to increase photon flux is to bind Fab fragments to ^{99m}Tc using bifunctional chelators. Weber et al. (*13*) compared ester linked to amide linked ^{99m}Tc-Fab′ antimyosin in mice and demonstrated similar biodistributions, but the ester linked conjugate had a twofold increase in renal clearance when compared to the amide linked complex, hastening blood-pool clearance. The improved renal clearance appears to be due to the fact that the ester bond undergoes hydrolysis in the kidney, producing byproducts that are easily excreted. This mechanism of renal hydrolysis is important since the kidney is the major organ for Fab′ antibody fragment catabolism and excretion. It was hypothesized that earlier imaging may be possible with ^{99m}Tc-RP-1 antimyosin.

The results from the present study showed that blood-pool clearance rates in primates for both agents were faster than the blood-pool clearance rate in humans for ^{111}In-Fab antimyosin (462 min versus 720 min). The resulting planar images of both agents were of good quality with demonstration of good graft uptake and good target-to-background ratios. Higher specific activity of ^{111}In-Pls produced target activity with lower doses of ^{111}In than for ^{111}In-Fab antimyosin. For both imaging agents there was a good general correlation between heart/lung ratios and biopsy scores. The higher ratios for the ^{99m}Tc-RP-1 scans probably reflect the superimposition of myocardial activity with some residual blood-pool activity at the earlier imaging times in addition to the higher dose of radiotracer given. Optimal imaging time for both agents appears to be at about 12–24 hr postinjection. Despite the relatively short half-life of ^{99m}Tc, counts were adequate at 12 hr due to the higher injected dose of radiolabeled antibody. However, based on the clearance data, residual blood-pool activity at 12 hr may interfere with assessment of diffuse myocardial uptake (Figs. 4 and 5). At 24 hr postinjection, insufficient technetium activity remains in the myocardium to image.

The only antibody modification that will significantly shorten blood clearance is to make the protein molecule smaller. Nedelman demonstrated fast blood-pool clearance and early infarct visualization in a canine infarct model using the sFv antimyosin fragment radiolabeled with ^{99m}Tc (*25*). The sFv fragment is a small molecule which comprises the variable regions of the Fab heavy and light chains and is produced by recombinant technology.

In summary, both antimyosin Fab agents demonstrated similar blood-pool clearance rates as slightly faster than values reported for ^{111}In-antimyosin in humans, but these rates were still not fast enough to allow imaging immediately or even soon (within 6 hr) after injection for detection of diffuse myocardial uptake seen in cardiac transplant rejection. Although faster than ^{111}In-Fab antimyosin, the blood-pool clearance of ^{99m}Tc-RP-1 antimyosin is not fast enough to guarantee low blood-pool activity, necessary to interpret diffuse uptake, before myocardial activity has decayed to levels too low to image. Of these two agents, ^{111}In-Pls antimyosin appears to offer some advantages over ^{111}In-antimyosin for clinical use in detecting cardiac transplant rejection, but whether the small benefits warrant clinical trials is unclear. The next step to overcome the major drawback of slow blood-pool clearance involves recombinant technology.

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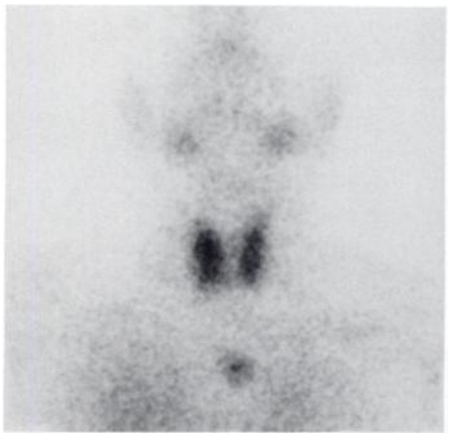

Figure 1. Thallium image.

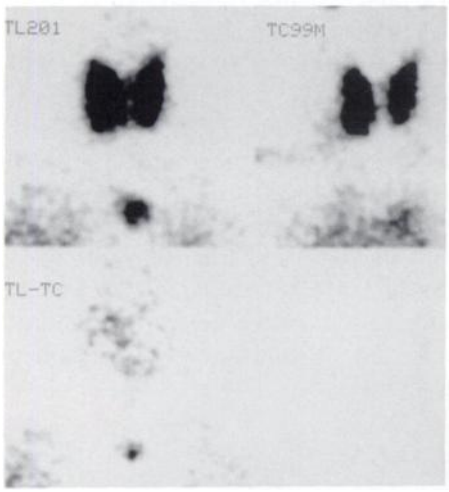

Figure 2. Thallium-technetium and substraction images.

FIRST IMPRESSIONS

PURPOSE

A 75-yr-old female with a history of unstable angina had an elevated calcium level of 12.9 mg/dl (normal 8.5–10.5). The parathyroid hormone level was 593 pg/nl (normal 10–65). A thallium/technetium parathyroid scan was performed and showed a focal lesion in the mediastinum consistent with parathyroid adenoma. The chest CT scan confirmed this finding. At surgery, a 2.5 cm mediastinal lesion was removed and identified as a parathyroid adenoma.

TRACER

^{201}Tl-chloride and [^{99m}Tc]pertechnetate

ROUTE OF ADMINISTRATION

Intravenous injection

TIME AFTER INJECTION

Immediately and 20 min

INSTRUMENTATION

Gamma camera (Elscint)

CONTRIBUTORS

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INSTITUTION

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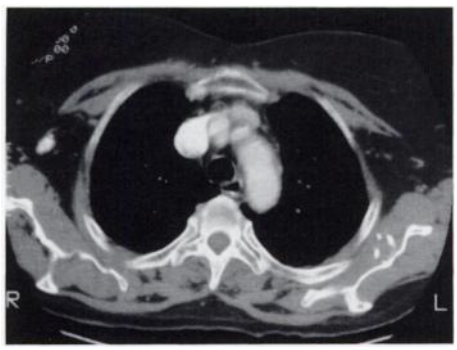

Figure 3. CT scan.