Detection of Deep Venous Thrombi and Pulmonary Embolus with Technetium-99m-DD-3B6/22 Anti-fibrin Monoclonal Antibody Fab' Fragment

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Technetium-99m-labeled anti-fibrin DD-3B6/22 Fab' monoclonal antibody fragments, which specifically target human cross-linked fibrin with high affinity, were evaluated in humans for safety and their capacity to detect deep vein thrombi and pulmonary embolism. **Methods:** Twenty patients with proven deep-vein thrombosis, documented by contrast venography, or venous duplex scan, were injected with a 600 MBq (0.5 mg) dose of antibody. Planar images of the lower limbs were recorded at 0, 2, 6 and 24 hr and chest scintigrams were recorded at 6 and 24 hr. **Results:** All venography documented thrombus sites, calves, popliteal and femoral, were detected with the radioimmunoconjugate. For the venous duplex scan-proven thrombus sites, all except two calf thrombi in two patients with bilateral disease and other positive sites were detected. Five patients had bilateral deep-vein thrombosis with multiple sites being visualized with the radioimmunoconjugate in the calf, popliteal and femoral regions. One case of pulmonary embolus was also definitively demonstrated. Documented thrombus sites were detected at 2 and 6 hr postinjection. Nineteen patients were on heparin. No adverse reactions to the injected dose were observed and one low titer human anti-mouse antibody response may have occurred. **Conclusion:** The results indicate that ^{99m}Tc-DD-3B6/22 Fab' has potential for noninvasive detection of deep-vein thrombosis and pulmonary embolism.

Key Words: technetium-99m; monoclonal antibodies; human cross-linked fibrin; deep vein thrombi; pulmonary embolism; contrast venography; venous duplex scan

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Venous thromboembolism is a major cause of morbidity and mortality. In Australia alone, 10,000 patients are estimated to die each year from pulmonary embolism (*1*). The concern for all clinicians who look after patients is that deep-vein thrombosis (DVT) is frequently not diagnosed before the occurrence of pulmonary embolism (*2*). This is due to the paucity of clinical symptoms in many cases and the significant limitations associated with currently available methods of investigation (*3*). Thus, the development of noninvasive techniques for the reliable detection of DVT and pulmonary embolism remains an important goal.

Cross-linked fibrin forms the underlying backbone of both venous and arterial thrombi (*4–6*). Thrombi are formed when the enzyme thrombin is activated, leading to the cleavage of fibrinopeptides A and B from soluble plasma fibrinogen (*7*). This initiates a reaction sequence which culminates in the local polymerization of fibrinogen into insoluble fibrin. In the later stages of polymerization, adjacent "D" domains of the rapidly forming polymer are covalently cross-linked. Cross-linked fibrin has a number of unique antigenic sites compared to the parent fibrinogen molecule (*8–12*). Monoclonal antibodies (Mabs) with specificity for the DD domain on cross-linked fibrin are therefore particularly promising as diagnostic agents since this epitope is unique to cross-linked fibrin, abundant throughout the anatomical extent of the thrombus and potentially accessible to an intravenously administered tracer due to the fact that thrombi are intravascular and undergo fibrinolysis.

The anti-fibrin Mab DD-3B6/22 has a high affinity for the DD domain of cross-linked fibrin and does not cross-react with fibrinogen, fibrinogen degradation products or fibrin monomer (*12,13*). Initial preclinical studies in rats and rabbits revealed excellent in vivo localization of ^{131}I-labeled intact antibody and F(ab')$_2$ fragments to both subcutaneous and intravascular DD-antigen implants (*13,14*). Subsequent experiments in rabbits and baboons, using a ^{99m}Tc-DD-3B6/22 Fab' immunoconjugate (^{99m}Tc-3B6), displayed

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TABLE 1
Clinical Findings and Investigations in 20 Patients with Proven DVT*

Patient	Sex	Age (yr)	Symptoms (days)	Venography	c	p	f†	Venous duplex scan	c	p	f	Impression of ^{99m}Tc-3B6 study	c	p	f	Plasma‡ D-dimer (ng/ml)
1	M	65	14	L	+	+	+	L	+	+	+	L	+	+	+	221
								R		o		R		+		
2	F	60	14	L	+	+	+	L	+	+	+	L	+	+	+	639
								R	+			R	+			
3	M	62	3					L	+	+		L	+	+		388
4	F	47	2	L	+	+	+	L	+	+	+	L	+	+	+	448
5	F	83	10					L	+	+	+	L	+	+	+	525
								R		o		R		+		
6	M	52	8					L	+	+	+	L	+	+	+	178
								R	+	+		R	+	+		
7	M	77	7					L	+	+	+	L	+	+	+	208
8	M	49	2					L	+	+		L	+	+		405
9	M	40	14					R	+	+	+	R	+	+	+	371
10	M	63	7	L	+			L	+			L	+			137
11	F	65	3	R	+	+	+	R	+	+	+	R	+	+	+	409
12	M	67	1					L	o	+	+	L	+	+	+	2847
								R	o	+	+	R	+	+	+	
13	M	60	3					L	+			L	+			363
14	M	54	3	L	+	+						L	+	+		363
15	F	81						R	+	+	+	R	+	+	+	901
16	F	68	7	R	+	+	+	R	+	+	+	R	+	+	+	326
17	M	66	12					L			+	L			+	377
18	M	50	14					L	+	+	+	L	+	+	+	549
19	M	60	3					R	+	+	+	R	+	+	+	383
								L	+			L	−			
20	F	77						R	+	+	+	R	+	+	+	241
								L	+	+	+	L	−	+	+	

*All patients on heparin except Patient 19.
†Calf (c), popliteal (p), femoral (f) regions.
‡Normal plasma D-dimer level <320 ng/ml.
+ is thrombus detected; o = calf or popliteal region not examined using venous duplex scan; and − is thrombus not detected using ^{99m}Tc-3B6.

high thrombus-to-blood ratios and clear gamma camera visualization of fresh and aged thrombi in the presence as well as the absence of heparin (*15,16*).

The aim of the clinical study reported here was: (1) to examine the localization capability of ^{99m}Tc-3B6 in patients with proven DVT, and (2) to assess its safety with particular reference to the development of toxic or allergic reactions. The conjugate was shown to detect DVT in all the patients examined, none of whom experienced any side effects.

METHODS

Patients

Twenty patients (13 male and 7 female) were recruited into the study. Their age range was 40–81 yr with a mean age of 62 yr (Table 1).

Patients were considered eligible for participation in the study if a DVT had been documented on contrast venography or venous duplex scan; they were at least 18 yr of age; and were available for follow-up over the ensuing 3 mo.

Any patient with a history of concurrent disease such as refractory epilepsy, severe uncontrolled hypertension with a diastolic blood pressure greater than 105 mmHg, or end-stage hepatic or renal failure was excluded from the study. Similarly, patients with clinical or biochemical evidence of disseminated intravascular coagulation or with a history of allergy to foreign animal proteins were also excluded, as were pregnant or breast-feeding women.

The protocol was approved by the Institutional Ethics Committee and informed consent was obtained from each patient.

Pulmonary embolism was confirmed by pulmonary angiography in Patients 13 and 19 who had high probability ventilation and perfusion scans.

Laboratory and Clinical Studies

Baseline studies were performed on all patients before scanning. These included chest x-ray, electrocardiogram and urinalyses, as well as biochemical, hematological, and coagulation profiles. The latter group of tests was repeated 1, 7 and 90 days after the injection of radioimmunoconjugate. A serum beta-hCG assay was carried out on females of childbearing age.

During the first 6 hr after intravenous injection of the radioimmunoconjugate, each patient's vital signs including temperature, pulse rate, blood pressure and respiratory rate were recorded on an hourly basis. Twenty-four-hour urinary collections were started from the time of injection in 13 patients. The anti-fibrin studies (vide infra) were performed within 3 days of documenta-

tion of the DVT except for Patient 20 (Table 1), where the interval between her venous duplex scan and ^{99m}Tc-3B6 study was 4 days.

Antibody Preparation

The production and properties of the murine Mab (DD-3B6/22) used in the study have been described previously (*12*). It belongs to the IgG3 subclass and is specific for the D-dimer of fibrin to which it binds with an affinity of $K_d = 2.7 \times 10^{-10} M$. Preparation of the $F(ab')_2$ and Fab' fragments was by standard techniques. The hybridoma cell line, the Mab-containing ascitic fluid and purified fragments were tested for sterility, pyrogenicity, cross-reactivity with male and female human tissue samples as well as for viral, mycoplasma and DNA contamination, according to the guidelines from the Commonwealth Department of Health, Housing, Local Government and Community Services for usage of Mabs in humans (*17*).

Antibody Labeling and Immunoreactivity of Conjugate

A modified Schwarz method (*15*) was used to label the Fab' fragments of DD-3B6/22 with ^{99m}Tc. Briefly, 1.2 mg of $F(ab')_2$ fragments of DD-3B6/22 were reduced to their Fab' fragments by incubation with an 80:1 molar excess of dithiothreitol. The Fab' fragments were then purified by centrifugal size-exclusion chromatography on Biogel PGD6 (Biorad, Richmond, CA), equilibrated in 0.1 *M* sodium acetate buffer, pH 5.6, before the addition of 2600 MBq of ^{99m}Tc-gluconate (Australian Radioisotopes, NSW, Australia) and incubated at 37°C for 30 min. The labeled protein was then purified as above and filtered through a 0.2-μ membrane filter (Millipore, NSW, Australia). Total recovered protein was measured by UV spectroscopy at 280 nm, and radioactivity was determined using a dose calibrator (Nuclear Associates, Carle Place, NY), allowing the specific activity (MBq/mg) to be calculated. All procedures were conducted within a biological safety cabinet class II (Gelman Sciences, NSW, Australia). The fraction of DD-3B6/22 Fab' fragments which retained immunoreactivity after ^{99m}Tc-labeling was estimated by means of a direct-binding assay to DD-Sepharose beads (Pharmacia, Uppsala, Sweden) as described previously (*14*). The conjugate was made up freshly for each patient, the immunoreactivity ranging from 65%–97%.

Gamma Camera Studies

On the day preceding the study, skin prick testing was performed with unlabeled $F(ab')_2$ fragments (1 mg/ml solution), to exclude the possibility of an allergic reaction to mouse protein. After placing the patient supine under the gamma camera, a single bolus intravenous injection of the conjugate (approximately 600 MBq of radioactivity and 0.5 mg of protein) was administered through a butterfly needle inserted into one of the cubital fossa veins.

Planar scans were obtained using a large field of view Philips Diagnostica 400 gamma camera, a low-energy, all-purpose collimator and computer collection on a PDP-11/73 computer. Anterior and posterior planar views of the calves, popliteal and femoral regions were obtained at time zero (dynamic and blood pool views), and then 2, 6 and 24 hr postinjection. Patients 4 and 7 (Table 1) had their studies at 0, 4–6 hr and 0, 1, 3, 5 and 24 hr respectively. Images were obtained using 5- and 10-min collections for the 2- and 6-hr postinjection studies respectively, whereas a 15-min collection time was needed at 24 hr. Anterior and posterior scans of the chest were also obtained at 6 and 24 hr postinjection. Anterior neck views of thyroid uptake were performed at 2, 6 and 24 hr in five patients. Patient management was not influenced in any way. In particular, heparin was administered in the usual dose and by the route of administration deemed clinically necessary.

The guidelines outlined by De Faucal (*18*) were followed for interpretation of scans based on the entire series of recorded images. Comparison of these sequential images with those obtained immediately postinjection allowed differentiation of thrombus localization from blood pool behavior. Other criteria included the development of asymmetrical uptake and the overall appearance of the tracer localization.

Venography

Venography of the leg suspected of having DVT was performed in the standard manner. Fifty to 100 ml of nonionic medium (Iopamiro 300; Schering AG, Germany) was manually injected under fluoroscopic control via a 22-gauge needle into a dorsal pedal vein. Tourniquets were placed at the ankle and above the knee. Spot film radiographs of the leg were obtained on a screening table in multiple projections, especially the antero-posterior and lateral views, with the patient semi-upright. Films of the pelvic veins and inferior vena cava were obtained with the patient supine after the tourniquets were released.

The diagnosis of a DVT was made according to the standard criteria of a filling defect within a deep vein and nonfilling of a deep vein with venous collateral formation.

Venous Duplex Scan

Venous duplex scans were performed with an Acuson 128 color-enhanced duplex scanner (Epping, Australia) (*19,20*). The patients were placed in a slight head-up position to allow venous pooling to fill the veins in the lower extremities. Care was taken to avoid postural compression of the veins. The proximal calf, popliteal, superficial and common femoral veins were imaged. Each segment was checked for compressibility and patency by intermittent transducer pressure and detection of venous flow. A diagnosis of DVT was made if intraluminal thrombus was imaged, veins were incompressible or no flow could be detected in the venous segment. Venous duplex scans were reported by one independent reader (JH).

Plasma Clearance

Serial blood samples were obtained from three patients (6, 16 and 18) at various time points from 5 min to 24 hr after injection to determine radioactivity clearance from the plasma. Data were analyzed assuming a two-compartment model of clearance (*21*).

Plasma D-Dimer Levels

Plasma D-dimer levels were determined with an enzyme immunoassay for cross-linked fibrin degradation products (Dimertest^R, AGEN Biomedical Ltd, Queensland, Australia), at preinjection, and then 1, 7 and 21 days poststudy. The upper limit of normal was 320 ng/ml (*22*).

HAMA Response

Human anti-mouse antibody levels to the ^{99m}Tc-3B6 injection were determined by a sensitive solid-phase, enzyme-linked immunoassay at preinjection and on Days 1, 7, 14 and 90 of the study based on a modification of the ENZYGNOST^R-HAMA micro (Behringwerke AG). The assay detected both human IgM or IgG responses to $F(ab')_2$ fragments of the DD-3B6/22 Mab coated to the wells of microtiter plates using a 1-μg/ml solution. Serial doubling dilutions of each plasma sample, commencing at 1/10, were tested in triplicate and end point titers (optical density < 0.1) were determined. A significant HAMA response in a given sample

was considered to have occurred if the end point titer of a postinjection plasma sample increased by two or more doubling dilutions, compared to the corresponding preinjection plasma sample. A normal range was obtained by determining the end point titers for IgG and IgM to DD-3B6/22 in 80 healthy blood donors.

RESULTS

Acute Toxicity Evaluation

No allergic or toxic reactions were observed. Monitoring of vital signs before and during the 6 hr following injection of the radioimmunoconjugate did not demonstrate any significant changes in systolic or diastolic blood pressure, heart rate, respiratory rate or temperature. There was no evidence of local erythema or discomfort at the injection site nor evidence of hypersensitivity reactions. Analysis of blood biochemistry and hematological data as well as coagulation profile and urinalysis results after ^{99m}Tc-3B6 injection did not reveal any significant changes from the preinjection state.

Clinical and Imaging Studies

A summary of the clinical findings and results of investigations in the 20 patients is presented in Table 1. Duration of symptoms of DVT varied between 1 and 14 days. Three patients (8, 12 and 18) gave a past history of DVT. Nineteen of the 20 patients were on heparin. Thyroid uptake was not significantly higher than the surrounding tissues in the five patients in which it was studied.

Thrombus sites were identified in seven patients using venogram results as the standard, and in the remaining 13 patients (and right calf in Patient 2) by venous duplex scan. Five patients had bilateral DVT. Eleven patients had left-sided unilateral disease and four patients had right-sided unilateral disease. Nine patients had unilateral DVT involving the whole lower limb (calf, popliteal and femoral) and six had localized unilateral DVT.

The results with ^{99m}Tc-3B6 demonstrated in vivo targeting of the immunoconjugate with focal, asymmetrically increased localization of the tracer being clearly recognizable at the site of known venous thrombi. Representative images shown in Figures 1–4 illustrate the essential features. Scintigrams of the left femoral region in Patient 7 revealed areas of irregular, relatively increasing, asymmetrical localization of the tracer over time. Localization was observed as early as 1 hr after injection (Fig. 1). At that time, tracer was apparent in the bladder because the radioimmunoconjugate is excreted mainly via the kidneys. The corresponding venous duplex scan was reported as showing a major axial venous thrombosis on the left side involving the superficial femoral vein to the level of the inguinal ligament. Scintigrams performed 6 hr postinjection were obtained from a male patient (Patient 18, Fig. 2) who gave a history of a past left calf DVT and had major axial vein thrombosis of the left leg extending from the calf to the common femoral vein on venous duplex scan. Asymmetrically increased tracer uptake was clearly demonstrated in the left femoral, popliteal and calf regions whereas the right

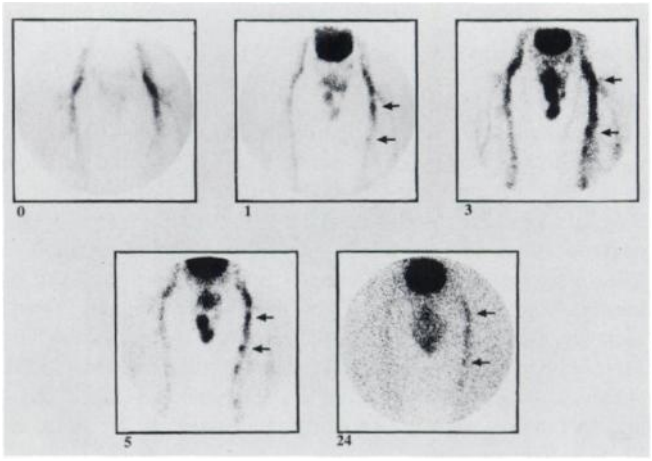

FIGURE 1. Anterior thigh views (Patient 7) taken at 0, 1, 3, 5 and 24 hr after the injection of ^{99m}Tc-3B6. Scintigrams indicate relatively increasing and irregular uptake in the left femoral vein (arrows).

leg was normal. The right leg was also examined ultrasonically and found to be clear of thrombus. Figure 3 illustrates posterior calf views of Patient 13 immediately and 6 hr postinjection. The scintigram shows readily recognizable localization of tracer along the left calf. On venous duplex scan, a left isolated peroneal vein thrombosis was detected. Scintigrams of the posterior right calf (Fig. 4) from Patient 11 taken 0 and 2 hr postinjection demonstrate relatively increased tracer accumulation, particularly in the popliteal region. A right-leg phlebogram, two days before the ^{99m}Tc-3B6 study, revealed extensive thrombosis in the popliteal, femoral and calf regions. The scintigram also demonstrated clear asymmetrical localization of the tracer in the femoral vein region (not shown).

These examples (Figs. 1–4) highlight a number of important points:

1. Early images, i.e., those taken within 10 min of ^{99m}Tc-3B6 injection, may differ appreciably in tracer distribution to those collected later (2, 6 and 24 hr) (Figs. 1, 3 and 4). Early images essentially showed blood-pool activity (*18*). With time, blood-pool activity decreased whereas the contrast between thrombus and background increased (Figs. 1, 2 and 3).

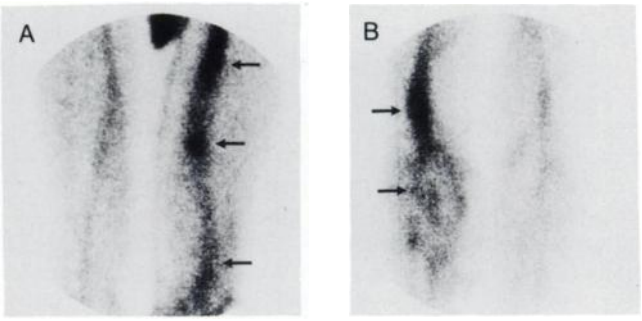

FIGURE 2. Scintigrams (Patient 18) of (A) anterior thigh and (B) posterior calf regions 6 hr after injection of ^{99m}Tc-3B6. There is asymmetrically increased tracer uptake demonstrated in the left femoral, popliteal and calf regions (arrows).

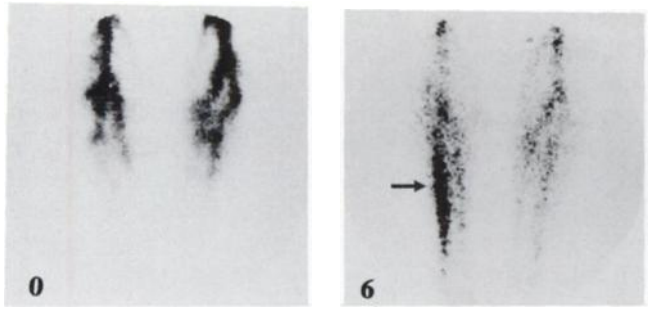

FIGURE 3. Posterior calf views taken (Patient 13) at 0 and 6 hr after the injection of ^{99m}Tc-3B6. Scintigrams show increased tracer accumulation in the peroneal vein region of the left calf (arrow).

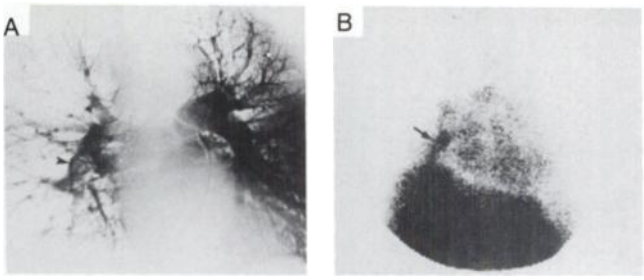

FIGURE 5. (A) Subtraction pulmonary angiogram (Patient 19) demonstrates a large filling defect (arrow) in the right pulmonary artery consistent with embolus. (B) Scintigram of anterior chest 6 hr after the injection of ^{99m}Tc-3B6 showing an area of increased uptake corresponding to the angiogram abnormality (arrow).

2. The availability of the whole series of images assisted interpretation. A region may exhibit blood-pool behavior over time or may show a relatively increasing tracer uptake consistent with thrombus localization (Figs. 1 and 3).
3. Good tracer localization to thrombus was detected in the studies done as early as 2 hr after injection in 12 of the 14 patients (excluding Patient 4), with unilateral disease, (Fig. 4). In bilateral DVT, although thrombi in one of the limbs was recognized at 2 hr, definitive diagnosis of thrombi bilaterally was achieved at 6 hr postinjection.
4. The 24-hr (15-min collection) images were particularly informative (Fig. 1). They were definitive for thrombus detection and confirmed the 2- and 6-hr results (Fig. 2).
5. Technetium-99m-3B6 was capable of demonstrating localized disease, i.e., in only the calf, calf and popliteal or just the femoral region. Figure 3, showing an isolated calf peroneal thrombosis, indicates that the technique can detect a thrombus of this volume.
6. Recurrent DVT, diagnosed in three patients including Patient 18 (Fig. 2), was readily identified using immunoscintigraphy.

Two patients had pulmonary emboli documented by angiography. The immunoscintigram (Patient 19) of the anterior chest taken 6 hr postinjection (Fig. 5) demonstrated an area of increased uptake, corresponding to the angiogram abnormality. The pulmonary angiogram was completed before starting the ^{99m}Tc-3B6 study. In Patient 13 (not shown), the angiogram demonstrated embolus in the right base. The scintigram revealed accumulation of tracer in the right lung very close to the liver uptake, which was suggestive but not diagnostic of pulmonary embolism.

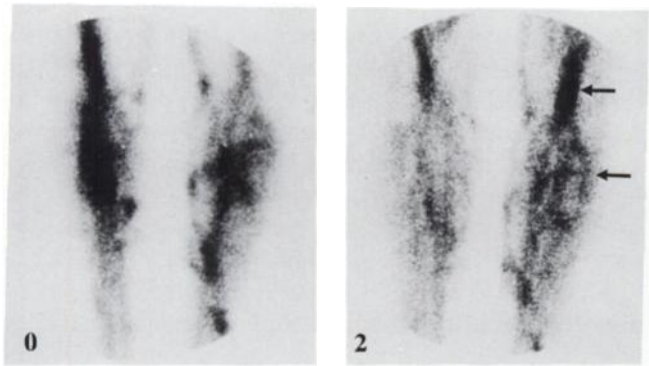

FIGURE 4. Posterior calf views (Patient 11) 0 and 2 hr after the injection of ^{99m}Tc-3B6. Scintigrams show popliteal and calf uptake (arrows).

Comparison with Venography and Venous Duplex Scan

All venogram-documented thrombosis sites (calf 7, popliteal 6, femoral 5) were detected with ^{99m}Tc-3B6. Immunoscintigraphy identified all duplex ultrasound-proven DVT sites (calf 15, popliteal 14, femoral 12) except for two in the calf. These occurred in two patients (Patients 19 and 20) who had bilateral disease with multiple sites. In patients with unilateral DVT, all documented sites were identified by 2 hr postinjection except for one calf, three popliteal and two femoral sites which were detected at 6 hr and those in one patient (Patient 4) whose initial images were not obtained until 4 hr postinjection. In the bilateral cases, all sites (calf 8, popliteal 8, femoral 7) were detected within 6 hr. There were six patients with localized venous thrombosis. In two patients, only the calf (Patients 10 and 13) was involved; in three patients, the calf and popliteal sites were involved (Patients 3, 8 and 14); and in a single patient (Patient 17), an isolated left common femoral vein thrombosis was present. These thrombi sites were identified using immunoscintigraphy with the other regions being negative.

The protocol did not specifically call for pelvic studies, however, nine sites were apparent in the iliac region despite interference from bladder activity. Only 1 patient had venography and this site did show thrombus. Venous duplex ultrasound showed six thrombi that extended above or to the inguinal ligament.

Although no normal subjects were included in the study, 24 sites (calf 5, popliteal 8, femoral 11) were examined with either venography or ultrasound and declared free of thrombus. These sites were also negative on immunoscintigraphy.

Laboratory Studies

Plasma D-dimer Levels. Individual values are summarized in Table 1. Five patients had D-dimer values over 500 ng/ml. The three highest values of 639, 2847 and 901 ng/ml occurred in terminally ill patients with disseminated carcinoma. No qualitative differences in the images from these patients were observed.

HAMA Response

Based on the normal range derived from the levels obtained in 80 blood donors, one patient demonstrated a mild IgG HAMA response with a twofold titer increase from 1/80 to 1/320, although there was no change in IgM titer, which remained at <1/10 throughout the testing period.

Plasma Clearance, Urinary Excretion and Dosimetry Considerations. Assuming a two-compartment model, curve-stripping analysis of the plasma clearance curves for ^{99m}Tc-3B6 indicated that the half-life of the fast component for three patients was 55, 56 and 75 min, while the half-life of the slow component was 8.4, 8.4 and 9.8 hr. The rapid phase contributed 32%–49% of the plasma clearance. The 24-hr urinary excretion rate from 13 patients ranged between 23%–72%. Immunoscintigraphy confirmed that the renal pathway was the normal route of excretion of ^{99m}Tc-3B6.

The biodistribution of ^{99m}Tc-3B6 was similar to that seen in previous preclinical studies in the baboon (*16*), where kidney uptake of 19% of the injected dose was observed within 1 hr after injection, with a slight fall to 17% at 24 hr. An administered activity of 600 MBq was used for the clinical studies to achieve images of adequate diagnostic quality. On the basis of measurements in the baboon, the corresponding absorbed dose to the kidneys from activity in the kidneys is approximately 55 mGy.

DISCUSSION

The studies described here demonstrate the ability of ^{99m}Tc-3B6 to target thrombus formed in vivo, the age of which encompassed a reasonable period of time on clinical grounds (one day to several weeks). Definitive localization and enhanced uptake was observed using gamma camera scintigraphy in all 20 patients with venous thrombosis documented by venous duplex scan and/or venography, even though 19 patients were receiving heparin at the time of the study. Tracer localization was comparable (Fig. 3) in location and extent to thrombus found on venous duplex scan or venography. In the images taken at 2 and 6 hr postinjection, excellent contrast between the target thrombus and nontarget tissue was achieved (Figs. 1–4). Recurrent DVT was readily detected.

Two false-negative results occurred in patients with bilateral DVT and multiple thrombus sites (Table 1, Patients 19 and 20) in the sense that a single site in each was not detected with the conjugate. There are three possible explanations for this finding. First, because of the time between the venous duplex ultrasound and the ^{99m}Tc-3B6 study (3 and 4 days, respectively), the calf thrombi may have resolved or become too small for detection. Second, identification of all thrombus sites may not have been achieved in the presence of bilateral DVT, because interpretation in this situation was more difficult. Third, bilateral disease represents a very large amount of thrombus in the venous system. All sites may not have been targeted because of a relative lack of tracer or lack of access. Whichever explanation is correct, however, the conjugate did detect other sites in the same two patients.

The study did not examine specificity or sensitivity formally because no patients without DVT entered the study by design. In the course of this study, 24 sites were found free of thrombus after venography or venous duplex scan examination. The same 24 sites did not show ^{99m}Tc-3B6 localization, which provided reasonable evidence for selective localization of ^{99m}Tc-3B6 to known thrombi.

No allergic or toxic reactions occurred and the HAMA response did not prove to be a problem with the labeled Fab′ fragment used here. However, the patients only received a single injection. The solution in the future could well lie in the development of humanized antibodies and even smaller fragments. Not only should humanization decrease immunogenicity, but production of even smaller fragments could enhance thrombus access and reduce the time to imaging by increasing the blood clearance rate (*23–27*).

Technetium-99m-3B6 possesses many of the required attributes (*3,25,28*) for successful radionuclidic detection of formed thrombus. Mab DD-3B6/22 very specifically targets the epitope at the DD site of cross-linked fibrin with known high affinity (*13*). It does not bind to circulating fibrinogen (*12,13*) or red cells and has not revealed cross-reactivity with other human tissues to date. Since cross-linked fibrin is the hallmark of formed thrombus, the targeting of the DD site identifies the presence of formed thrombus and, as a corollary, a lack of cross-linked fibrin would imply a lack of formed thrombus. The DD sites on cross-linked fibrin appear rapidly from the onset of thrombogenesis (*7*) and remain associated with the core of the fibrin clot until lysis is complete (*7,29*), allowing the detection of "fresh" and older formed thrombi (*13*), including those in recurrent DVT. Formed thrombus offers many potential binding sites, since DD is the repetitive structural component of cross-linked fibrin present throughout its anatomic extent. Consequently, the targeting of this epitope by DD-3B6/22 has the potential to be a sensitive, as well as a specific test for formed thrombus, which may be a significant factor in situations where sensitivity of detection is limiting (*27,30*). According to the results reported here, binding occurs rapidly and is sufficiently stable for successful imaging with the gamma camera.

Although DD-3B6/22 does not react with fibrinogen, fibrinogen degradation products nor fibrin monomer, it does bind to cross-linked fibrin degradation products. Previous animal data indicated that localization or scintigraphic visualization of the thrombus was maintained in the presence of D-dimer titers of 8.9 μg/ml (*11,31*). This level is well in

excess of the highest values obtained here and the reported mean levels of D-dimer in patients suffering from DVT (1.096 μg/ml) or pulmonary embolism (1.25 μg/ml) (*32*). Levels of this order of magnitude (8–9 μg/ml) are seen only in patients with severe disseminated intravascular coagulation (*32*). Therefore, the amount of circulating D-dimer expected in patients with DVT is unlikely to significantly affect, at a clinical level, the localization ability of ^{99m}Tc-3B6. This conclusion is supported by the findings here.

The value of Mab (*8–12,23,33*) directed against other components of coagulation pathology (i.e., beta chain of fibrin, platelets), is also being investigated for in vivo thrombus imaging by gamma camera detection. The Mab DD-3B6/22 represents a viable alternative to these antibodies for several reasons; first, the DD region of cross-linked fibrin is not readily lost upon initial fibrin degradation and may be more accessible to the antibody fragment (*34*). Second, targeting the DD site may be more successful in imaging formed older thrombi (*11,13,35*). Third, antibodies which recognize the DD site of cross-linked fibrin would, theoretically, be less susceptible to interference by heparin (*11,35*). Fourth, antibodies reactive with platelets (*33*) are influenced by heparin and only detect actively forming or fresh thrombi (less than 2 days old) (*3,35*). In contrast, ^{99m}Tc-3B6 localizes and delineates both fresh and older formed thrombus, to which platelets no longer aggregate (*36*). By choosing this antibody, it was anticipated that the conjugate would be retained for a much longer time in the vicinity of the clot. Thus, targeting the specific epitope in the DD site which remains associated with the fundamental structure of the fibrin clot until complete lysis is clearly advantageous.

CONCLUSION

Technetium-99m-DD-3B6/22 Fab′ targets the DD site on cross-linked fibrin, a specific marker of formed thrombus with high affinity ($K_d = 2.7 \times 10^{-10}$ *M*). No allergic or toxic reactions were observed in the 20 patients studied. Excellent contrast images of venous thrombi were obtained at 2 and 6 hr postinjection. All sites of venous thrombi, except for one in the calf in two patients each with bilateral disease and other positive sites, were identified as well as an incidental pulmonary embolus. Nineteen of the 20 patients were on heparin. This radioimmunoconjugate has potential for the gamma camera detection of pathological cross-linked fibrin in the body, for example that associated with some tumors but, in particular, for the detection of formed venous thrombi and pulmonary embolus.

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EDITORIAL

Thrombus-Specific Imaging: Approaching the Elusive Goal

The importance of developing a thrombus-specific imaging method is a goal of undisputed clinical importance. Whereas thrombus formation after vascular injury is cardinal in hemostasis, it is also the cause of unwanted effects due to occlusions and emboli in the arterial and venous circulation. Early detection and treatment of pathological intravascular thrombosis is effective. The high incidence of side effects from thrombolytic therapy, however, necessitates accurate identification of patients who will not benefit from such treatment.

A noninvasive, rapid and specific method for thrombus detection which will also enable monitoring thrombus dissolution is therefore highly desirable. Such a method should preferably allow imaging of arterial and venous thrombi of various ages and help determine whether a thrombus is still amenable to thrombolytic therapy. This topic has been reviewed intensively, including mesenteric ischemia and thrombolysis (*1–6*).

The search for thrombus-specific imaging agents began two decades ago when radioiodinated fibrinogen was first evaluated (*7*). Since then, a plethora of thrombus imaging agents have been described, including agents that are incorporated into thrombi and agents that bind to components of previously formed thrombi (*7–29*).

Radiolabeled platelets (*8*) and antiplatelet antibodies will bind to forming thrombi (*14,15,19*), antifibrin antibodies (*16,20–23*), anti-activated platelet antibodies (*24,25*) and active or inactivated tissue type plasminogen activator (t-PA) (*26–28*) will bind to formed, older thrombi.

Theoretically, agents targeted to forming thrombi should be suitable for imaging fresh propagating thrombi. In addition, an agent may show preferential binding to a thrombus type based on the abundance of that specific factor in either arterial or venous thrombi. Thus, radiolabeled platelets or antiplatelet antibodies will display higher affinity for arterial thrombi that are rich in platelets, while venous preformed thrombi will probably be better imaged with agents that bind to fibrin, antifibrin antibodies or by native or inactivated t-PA.

Although venous thrombosis and embolism occur frequently, resulting in a high incidence of morbidity and mortality, arterial thrombosis is of greater concern. Thrombus formation on atherosclerotic plaques may result in complete occlusion of coronary arteries, leading to critical and often fatal infarction. Although imaging arterial thrombosis is of great importance, little success has been achieved to date (*14,15,29–31*). This disappointing situation may have resulted from the use of compounds with inadequate thrombus-to-blood ratios and small-size arterial thrombi, particularly in the coronary circulation.

The greatest success has been achieved in imaging venous thrombosis with monoclonal antifibrin antibodies. The reports in the literature include antibodies that react with the NH_2 group on the alpha (*32*) or beta terminus (*16,33–34*), on the D-domain of the noncrosslinked fibrin (*35*), on the fibrin D-domain after plasmin digestion (*36*) or on cross-linked DD-dimer regions (*37*). In the current issue of the *Journal*, Bautovich et al. report human studies using an antifibrin antibody directed against cross-linked human fibrin dimer (*38*). Bautovich et al. offer convincing evidence of the safety, feasibility and efficacy of the monoclonal antifibrin antibody method. By using a Fab' fragment preparation labeled with ^{99m}Tc, thrombus imaging was accomplished 2–6 hr after injection, a significant improvement over previously reported compounds that required 6–24 hr of preparation before successful imaging. Moreover, this method demonstrated pulmonary emboli. False-negative

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