Noninvasive Arterial Thrombus Imaging With 
$^{99m}$Tc Monoclonal Antifibrin Antibody

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Background. The T2G1s monoclonal antifibrin antibody binds specifically to fibrin but not to fibrinogen.

Methods and Results. In a canine model of acute arterial thrombosis, we determined the feasibility of imaging thrombi using a $^{99m}$Tc-labeled Fab' fragment. In 14 dogs, 10 carotid and 13 femoral artery thrombi were produced using 2-hour temporary occlusion, crush injury, and local thrombin injection methods. A sham-operated carotid artery served as control. Antifibrin antibody was injected intravenously at the end of temporary occlusion. Serial planar radionuclide images were obtained immediately and at 1 and 2 hours. Following killing the dogs at 2 hours, we measured antibody uptake ex vivo in 5-mm-long segments of thrombus, the adjacent injured artery, and a control artery. Antibody was cleared from the blood with a mean±SD t$_{1/2}$ of 121±23 minutes. The thrombi weighed 218±140 mg. Antibody uptake in the thrombi was patchy, and the thrombi were closely adherent to the injured arterial wall. In the segment with maximal ex vivo antibody uptake, the ratio of control artery to blood counts/g/sec was 0.65±0.46, the injured artery-to-blood ratio was 2.35±1.01 (p < 0.0001 versus control), and the thrombus-to-blood ratio was 4.24±2.58 (p < 0.0001 versus control). In three dogs, an isotype-matched ovarian tumor antibody labeled with $^{111}$In was injected with T2G1s but was not taken up in the thrombus or the adjacent arterial wall. Visual analysis of the in vivo carotid radionuclide images showed uptake by 2 hours in all 10 carotid thrombi. Quantitative image analysis, measured as the thrombus-to-opposite carotid artery ratio, showed increasing uptake over time with ratios of 1.1±0.3, 1.6±2.0, and 2.2±1.3 on the immediate, 1-hour, and 2-hour images, respectively. All quantitative ratios of 1.3 or greater were visually identified.

Conclusions. $^{99m}$Tc-labeled Fab' fragments of the T2G1s antibody are taken up specifically by acute arterial thrombi after intravenous injection. Uptake is progressive over a 2-hour period, and all thrombi are detected by radionuclide imaging at 2 hours. These results show that it is feasible to noninvasively detect arterial thrombi within 2 hours of formation. (Circulation 1992;85:298–304)

Arterial thrombosis is a causative factor in unstable angina, acute myocardial infarction, sudden cardiac death, transient ischemic attacks, and stroke.1–3 The ability to noninvasively localize thrombi could have widespread application in the diagnosis and treatment of these disorders. All existing noninvasive imaging techniques for thrombus detection have significant limitations. Anatomic methods, such as ultrasound and radiographic computed tomography, do not provide physiological information on the activity of a detected thrombus.

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Radionuclide methods using labeled platelets provide physiological information, but platelet labeling is time consuming and technically complex, and studies are usually not positive until 24–48 hours after isotope injection.4 A novel imaging approach is the use of specific antibodies directed at various thrombus components. One such antibody, T2G1s, reacts specifically with the first seven amino acids of the fibrin β-chain.5 A similar antifibrin antibody, 59D8, react with the same por-
tion of the β-chain. In humans and dogs, T2G1s binds to fibrin in areas of active thrombosis but not to the fibrinogen throughout the blood pool. The use of this antibody for imaging has been limited by a slow blood clearance and the poor imaging characteristics of \(^{111}\)In and \(^{99m}\)Tc used for labeling.\(^5-13\) Recently, murine monoclonal Fab' fragments of the T2G1s antifibrin antibody, which are cleared more rapidly from the blood than whole antibody, have been produced and labeled with \(^{99m}\)Tc, a radioisotope with desirable imaging properties.\(^14,15\) Venous thrombi in human limbs have been imaged after intravenous administration of antifibrin antibodies.\(^16,17\) Unlike venous thrombi, which contain relatively large concentrations of fibrin, arterial thrombi consist mostly of platelets with some admixed fibrin.\(^18\) For this reason, it is unclear whether T2G1s will be useful for imaging arterial thrombi.

To determine the usefulness of \(^{99m}\)Tc-labeled T2G1s Fab' for the detection of acute arterial thrombi, we used a canine model of fresh arterial thrombosis to investigate the ex vivo binding characteristics and the in vivo detection and time course of uptake using radionuclide imaging.

**Methods**

**Thrombosis Model**

All animal and experimental and surgical procedures were approved by the Animal Care Committee of the Seattle Department of Veterans Affairs Medical Center. In 14 dogs sedated with sodium thiopental and anesthetized with 1–2% halothane, arterial clot was induced in a 4–5-cm segment of a carotid artery \((n=1)\), a femoral artery \((n=4)\), or both \((n=9)\). Blood gases, arterial pressure, and heart rate were monitored and maintained in the physiological range. The artery was temporarily ligated and subjected to external crush injury using forceps, and 100–200 units thrombin was injected locally. The proximal ligature was released, and the artery was allowed to fill with blood. After the ligature was retyed, the occlusion was maintained for 2 hours. This method has been used by us extensively and produces occlusive thrombi in more than 90% of arteries.\(^19,20\) In three dogs, Doppler flow probes were applied distal to the thrombus, and there was no evidence of blood flow throughout the course of the experiment. Thirty seconds before releasing both the proximal and distal ligatures, \(^{99m}\)Tc T2G1s was injected intravenously. In three dogs, a non-fibrin-specific, isotype-matched monoclonal antibody (OV-TL3, specific for ovarian tumor–associated antigen; donated by Centocor, Inc, Malvern, Pa.) was labeled with \(^{111}\)In and coinjected with the antifibrin antibody.\(^21\) A surgically exposed but uninjured carotid artery served as a control vessel. Dogs were killed upon completion of the protocol by injection of potassium chloride.

**T2G1s Radiolabeling With \(^{99m}\)Tc**

T2G1s Fab' was prepared from immunoglobulin G produced by murine hybridoma methods.\(^14\) Radiola-

- **Radionuclide Imaging**

  Studies of the femoral and carotid regions were acquired using a large-field-of-view gamma camera equipped with a general, all-purpose parallel hole collimator and interfaced to a dedicated computer system. Immediately before injection of \(^{99m}\)Tc T2G1s, \(^{57}\)Co markers were placed at the side of the proximal and distal ligatures, and images acquired to identify the location of the thrombus and the dog and camera were stationary throughout the remainder of the experiment. \(^{99m}\)Tc or \(^{111}\)In images were acquired immediately and at 1 and 2 hours after intravenous antibody injection. All studies were acquired in the anterior projection using a 64×64 image matrix with ±20% energy windows centered on 122 keV for \(^{57}\)Co, 140 keV for \(^{99m}\)Tc, and the 247- and 173-keV peaks for \(^{111}\)In. Total counts over the entire field of view for the 2-minute \(^{99m}\)Tc acquisitions ranged from 700,000 to 2 million.

- **Ex Vivo Processing**

  The injured artery containing the thrombus and an equal-sized segment of the control carotid were excised, the proximal and distal ends were marked, the vessel was opened longitudinally, and the entire specimen was fixed overnight in formalin. Thrombus was separated from the arterial wall, and the specimens were sliced into numbered 5-mm-long segments, individually weighed, and well counted. Segments and whole blood were well counted for 1 minute on a Packard Minaxe Autogamma-5000 gamma counter with correction for background activity. Appropriate corrections for \(^{111}\)In downscatter...
into the \(^{99m}\)Tc window were made in specimens into which \(^{111}\)In and \(^{99m}\)Tc antibodies were injected. The thrombus and vessel wall were closely adherent, and upon separation, portions of the thrombi remained attached to the wall. Uptake of labeled antibody was expressed in counts per gram per second of tissue and normalized to whole blood at the time of death.

To quantify uptake of the labeled antibody on the well-counted specimens, we calculated the following ratio:

\[
\text{Artery or thrombus-to-blood ratio} = \frac{\text{counts/g/sec of artery or thrombus}}{\text{counts/g/sec of whole blood at death}}
\]

Because whole blood is always contiguous to thrombus in vivo and isotope is present in blood for a variable time period, this is an important index for determining feasibility of detection by external imaging.

Because antibody uptake was not uniform throughout the length of the large preformed thrombus or arterial wall, we calculated a maximal uptake ratio for the single artery or thrombus segment with the highest uptake as well as the average ratio, which was the mean value of all of the 5-mm-long segments of artery or thrombus.

**Histological Tissue Preparation**

Eight injured arteries (four femoral and four carotid) and 10 thrombi (five femoral and five carotid) were processed for histological sectioning. The 5-mm-long segments of wall and thrombi were embedded in paraffin, and a representative 6-mm-thick section, oriented perpendicular to the long axis of the vessel, was stained with hematoxylin and eosin. Each was reviewed by two independent observers under light microscopy, and the proportion of red blood cells and platelets present in the thrombus was visually estimated. The average of the two observers was used.

**Qualitative Image Analysis**

Each of the carotid radionuclide images was read independently by two experienced observers. The thrombosed carotid artery was compared with the opposite normal vessel and scored using the following system: −1, less than the normal vessel; 0, same as the normal vessel; 1, slight increase relative to the normal vessel; 2, moderate increase relative to the normal vessel; and 3, marked increase relative to the normal vessel. The mean value of the two scores was used for analysis. The femoral thrombi had visually increased uptake relative to the adjacent areas of normal vessel, but analysis was not possible due to increased uptake of antifibrin at the insertion site of an arterial line in the opposite femoral artery. In addition, blood pool activity in the penis and urine activity in the urethra and bladder made visual interpretation difficult. Thus, only well counting and histology results are reported for the femoral thrombi.

**Quantitative Image Analysis**

Using the cobalt marker images, the length of the injured artery on the \(^{99m}\)Tc antibody images was identified visually, and the area containing the maximal activity was marked. Next, a 1-pixel-thick line was drawn through the area of maximal activity and extended to the opposite normal carotid. An activity profile for counts per pixel was generated, the peaks corresponding to the thrombosed and normal carotid arteries were identified, and the ratio of maximal uptake in the damaged to normal carotid was calculated.

**Statistical Analysis**

All values are expressed as the mean±1 SD value in the text and plotted as the mean±SEM value in the figures. Ex vivo uptake in the injured artery and thrombus were compared with the control artery, and differences were determined using two-tailed, unpaired \(t\) tests. \(p<0.05\) was considered statistically significant.

**Results**

T2G1s was cleared from the blood with a calculated half-life of 121±23 minutes, with a range of 87–168 minutes (\(n=11\)). Three representative dogs are shown in Figure 1. Between the initial blood sample at 5 minutes and the final predeath blood sample at 2 hours, there was a 54% reduction in total counts.

**Histology**

The average weight of the 23 arterial thrombi was 218±140 mg and ranged from 36 to 553 mg. The 10 carotid thrombi weighed 269±59 mg (range, 87–553 mg), and the 13 femoral thrombi weighed 179±41 mg (range, 36–439 mg). The weight difference between the carotid and femoral thrombi was not statistically significant (\(p=0.13\)).

The thrombi were predominately of the red cell type. The majority of samples consisted of 90–95% red cell thrombi; the remainder were platelet thrombi. In a few samples, 30–50% of the thrombus...
mass consisted of platelet aggregates. Shown in Figure 2 is an example of a typical thrombus. The darker areas are red cell thrombus, and the paler lines of Zahn represent platelets with fibrin. The arterial walls showed hemorrhage into cleftlike spaces of the media and thin layers of red cell thrombi adherent to the intima. These changes were most likely caused by the crush injury.

**Ex Vivo Well Counting**

The variability in T2G1s uptake shown in the histological sections is reflected in the well-counting data. Figure 3 shows the results from a single dog of a well-counted femoral thrombus sectioned into 5-mm-long segments. The proximal segment has the greatest uptake, the four central segments have uniformly low uptakes, and the distal segment shows a slight increase. In general, for all thrombi examined, uptake was greatest at the proximal and distal ends of the thrombus, but there was considerable variability.

Figure 4 shows the ex vivo thrombus– or artery–to–whole blood uptake ratios of the average and maximum segments for 13 control arteries, 23 injured arteries, and 23 thrombi. The control artery–to–whole blood ratio was 0.41±0.29 for the average of all segments and 0.65±0.46 for the segment with

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**Figure 2.** Photomicrograph of hematoxylin and eosin–stained sections of a recently formed femoral artery thrombus. Darker areas consist mainly of red blood cell aggregates, and lighter areas (lines of Zahn) consist predominately of platelets and strands of fibrin. We estimated that 90% of this thrombus consists of erythrocytes. Magnification, ×60.

**Figure 3.** Bar graphs of T2G1s uptake (counts/sec) in a femoral thrombus from a single dog shown for each of six 5-mm-long segments. Uptake is heterogeneous and highest in proximal segment, with distal segment having a slight increase relative to four middle segments.

**Figure 4.** Bar graphs of artery– or thrombus–to–blood ratios (mean±SEM) shown for control artery (n=13), injured artery (n=23), and thrombus (n=23), using average uptake for all 5-mm segments or segment with maximal uptake of T2G1s. Uptake was significantly higher in injured artery and thrombus relative to control artery using either average or maximum counts, but maximum counts gave highest ratios.
maximal uptake, indicating less activity in the control artery than in whole blood at the time of death. The injured arterial wall took up more $^{99m}$Tc T2G1s than the control artery as reflected in the artery–to–whole blood ratio for the average segment (1.42 ± 0.62, \(p < 0.002\)) and for the maximal segment (2.35 ± 1.01, \(p < 0.0001\)).

The thrombus–to–whole blood ratio was 2.13 ± 1.80 for the average segment (\(p < 0.0001\) versus control artery) and 4.24 ± 2.58 for the maximal segment (\(p < 0.0001\) versus control artery).

Artery- or thrombus-to-blood ratios for the $^{111}$In-labeled OV-TL3 antibody were low using the average or maximal counts; for the control artery, injured artery, and thrombus, ratios were 0.25 ± 0.12 (\(n = 3\)), 0.55 ± 0.06 (\(n = 5\)), and 0.42 ± 0.17 (\(n = 5\)) using average and 0.35 ± 0.09, 0.70 ± 0.11, and 0.56 ± 0.27 using maximal uptake, respectively.

### Imaging Data (Carotid Arteries)

Figure 5 shows the mean visual and quantitative ratios for the two observers on the immediate and 1-hour and 2-hour carotid images. There was exact scoring agreement between the two observers in 22 of 30 vessels scored and disagreement by only one grade in the remaining eight. There was slight uptake in three dogs immediately after injection. By 1 hour, all 10 thrombosed vessels had slightly-to-markedly increased uptake (mean score, 1.7 ± 0.9), and at 2 hours, the mean uptake score was 2.4 ± 0.7. At 2 hours, a score of 2 (moderate uptake) or 3 (marked uptake) was present in nine of 10 dogs. The dog in Figure 6 had little T2G1s uptake on the immediate images but had marked uptake at 1 and 2 hours.

As shown in Figure 5, the uptake of T2G1s into the carotid thrombi, as measured by a quantitative ratio that compared the thrombosed artery with the contralateral control artery, was time dependent. The mean quantitative thrombus–to–normal vessel ratios were 1.1 ± 0.3, 1.6 ± 0.5, and 2.2 ± 1.3 on the immediate, 1-hour, and 2-hour images. Ratios of 1.3 or greater were associated with visually positive images, and this value was achieved by 2 hours in every dog.

### Discussion

In a canine model of acute arterial thrombosis, we have shown that $^{99m}$Tc T2G1s Fab' administered intravenously after thrombus formation is cleared from blood and taken up specifically in sufficient quantity to allow noninvasive radionuclide imaging 2 hours after injection.

Prior studies involving imaging thrombi with antifibrin antibody have been limited by using polyclonal
or whole antibodies and labeling the antibody with $^{131}$I or $^{111}$In. Polyclonal antibodies react nonspecifically with fibrin and fibrinogen, and whole antibody is cleared very slowly from blood due to minimal renal filtration.\textsuperscript{7,8} This results in persistently high background activity due to both free and fibrinogen-associated labeled antibody. Under these circumstances, noninvasive radionuclide imaging was possible only at 24–48 hours to allow adequate background clearance and uptake by thrombi. This required the use of long half-life isotopes such as $^{131}$I or $^{111}$In. Although antibody labeling with these radioisotopes is technically simple, the high-energy, low-photon release results in poor image quality.

Refinements in the production of monoclonal anti-fibrin antibody and the use of antibody fragments rather than whole antibody optimize specificity, blood clearance, and penetration into the target area and make it practical to consider imaging thrombi within several hours of their formation. Two monoclonal anti-fibrin antibodies, 59D8 developed by Hui et al.\textsuperscript{9} and T2G1s developed by Kudryk et al.,\textsuperscript{6} have high specific affinity for the NH$_2$ terminal portion of the $\beta$-chain of fibrin II. Because these antibodies have similar high-affinity constants for the same epitope, they are considered comparable.\textsuperscript{15} They do not cross-react with fibrinogen and overcome the specificity problem encountered with the use of polyclonal antibodies. F(ab')$_2$ and Fab' fragments, which have higher renal filtration and better penetration into the site of thrombus formation, have been produced to facilitate blood clearance and further improve thrombus-to-blood ratios. However, the 2-hour half-life is still long, and more rapidly clearing platelet- or fibrin-specific targeting agents are needed. With these two anti-fibrin antibodies in animal models, venous thrombi created in vivo can be noninvasively imaged at 24–48 hours, and ex vivo thrombus-to-blood ratios of 7–10:1 are present.\textsuperscript{9–12} However, in these studies, imaging was still performed at 24–48 hours using $^{131}$I or $^{111}$In to obtain optimal detection of thrombi. Human studies using $^{111}$In labeling have also shown that imaging of venous thrombosis was possible.\textsuperscript{16,17} Studies in the lower extremities frequently became positive within the first few hours after intravenous injection due to minimal surrounding blood pool activity. Because venous thrombi differ from arterial thrombi in the relative percentages of erythrocytes, platelets, and fibrin, the imaging results from these studies may not be applicable to arterial thrombi.\textsuperscript{18,22}

Radiolabeling the T2G1s Fab' fragment with $^{99m}$Tc provides optimal imaging energy and count rate using this widely available isotope. Although $^{99m}$Tc labeling of proteins has been technically difficult, recent advances have made it possible, and all of our studies were performed using an instant kit.\textsuperscript{15} We used $^{99m}$Tc-labeled Fab' fragments of the anti-fibrin antibody to image thrombi in an animal model that mimics the clinical setting of acute arterial thrombosis.\textsuperscript{23,24} Imaging was performed only over the first 2 hours to reflect a realistic time frame for the identification of and/or possible monitoring of therapy for clinical arterial thromboses. Our data show that in this model, identification of thrombus within 2 hours was easily accomplished. The thrombi in our model were created and present in arteries with an initially high flow rate; however, the necessity of temporary occlusion to reliably produce thrombi presumably makes them somewhat different from arterial thrombi formed in pathological states.\textsuperscript{23–25} Histologically, these thrombi were mixed platelet and red cell thrombi, with red cell thrombi predominating. In occlusive coronary thrombi in humans, the proximal portion is predominately platelets, with the majority of downstream material of the red cell type. Alternatively, in one animal model of coronary reocclusion, platelet thrombi predominate.\textsuperscript{24} In clinical syndromes of arterial thrombosis, it is uncertain whether sufficient red cell fibrin thrombi might be present to be detected by antifibrin imaging.

In the present study, we created thrombi in peripheral arteries. Radionuclide imaging is optimized in these arteries in that with total occlusions, blood activity is present only at each end of the occlusion, with the contrast with surrounding tissue relatively high. In the coronary arteries, a smaller-diameter clot is formed, and it always overlaps or is very close to the central cardiac blood pool activity. Even with the relatively short (121 ± 23 minutes) half-life of T2G1s, this blood pool activity may limit identification of thrombi formed in the coronary or pulmonary arteries. It is possible that late imaging, when blood pool activity has diminished, may allow imaging of these central thrombi. In a pilot study of coronary occlusive thrombosis in two dogs, we failed to detect thrombus by external imaging after intravenous injection of antifibrin antibody despite thrombus-to-blood ratios of 13:1 and 22:1 (unpublished observations). Our model produces total occlusions in which antibody was predominantly taken up only at the active ends. In clinical situations, incomplete occlusion and/or reperfusion might expose a greater surface of thrombus to antibody, producing relatively greater uptake.

In summary, in an animal model of acute arterial thrombosis, we have demonstrated avid uptake of labeled antifibrin antibody. This uptake is seen within a sufficiently rapid time frame to be appropriate for the diagnosis and/or possible monitoring of interventions in the clinical setting of acute arterial thrombosis. Whether these findings can be applied to the clinical setting now appears to deserve study, with thrombi in peripheral arteries being those most likely to be easily identified.

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