Editorial: Of Monoclonal Antibodies and Thrombus-Specific Imaging

The diagnosis of deep vein thrombosis (DVT) and pulmonary embolism (PE) is far from ideal. The number of patients with diagnosed PE compared to the high number of cases diagnosed only at autopsy does not seem to have changed in the last decades. On the other hand, indiscriminate, preventive anticoagulant therapy is not a feasible alternative for solving this problem because of the high rate of complications related to anticoagulation. Clearly, better ways of diagnosing these conditions are important.

Arterial thrombosis and embolism, mostly secondary to atheromatosis, is even more difficult to diagnose noninvasively. Although progress has been made by using NMR imaging and other noninvasive modalities, the mainstay in routine clinical diagnosis remains contrast radiographic techniques. The introduction of bypass coronary grafting, balloon dilation of narrowed blood vessels, thrombolytic, and anti-platelet therapy require repeat, serial evaluations of the treated stenosed vessels, given the high rate of re-stenosis reported when using these modalities. The efficacy of thrombolytic therapy is hindered by bleeding complications occurring mostly at "access" sites—cutdowns performed for catheter placement.

Two major approaches aimed at improving the noninvasive diagnosis of thromboembolic phenomena have evolved in the last decade. Both approaches are based on the use of radiolabeled monoclonal antibodies for noninvasive thrombus imaging: one uses anti-platelet antibodies, the other monoclonal antibodies targeted to fibrin. Platelets and fibrin are major constituents of thrombus, and imaging based on their detection may be considered thrombus-specific, that is, tests will be positive only when a vessel is occluded by thrombus. In this situation, anticoagulation and/or thrombolytic agents would be expected to open the occlusion.

Ideally, an antibody-based, thrombus-specific agent should have the following characteristics:

1. Exclusive thrombus-specific binding sites with no epitopes on circulating blood components or other tissue.
2. The binding on thrombus should be of high affinity, high density, with fast antibody binding.
3. The antibody binding sites should be stable and should not be affected by thrombus maturation, degradation, or anticoagulant therapy.
4. Fast blood disappearance of unbound antibody, to increase thrombus to background contrast.
5. Low or nonimmunogenic, to enable safe repeat examinations.
6. The antibody should preferably be available as an instant kit for technetium-99m labeling.
7. Meaningful imaging should be possible within 1–2 hr after injection of labeled antibody.
8. Imaging in low and high blood-pool organs should be feasible.

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THE ANTI-PLATELET ANTIBODY APPROACH

The first report on successful thrombus radioimmunoimaging was published in 1985 (*1*). The antibody against human platelets (7E3), labeled with indium-111 (^{111}In) or iodine-123 (^{123}I), enabled imaging of experimental venous and arterial thrombi in the limbs, neck, lungs and heart 1.5–2.0 hr after injection. The antibody 7E3, binds to the IIB/IIIA glycoprotein complex, a major component of the platelet membrane. The good clot-to-blood ratios obtained with 7E3 resulted from the high affinity of the antibody to resting (circulating) and activated platelets (platelets in thrombi) and to the rapid clearance of the antibody from the blood (in 20–30 min, the initial blood levels were reduced to 50%). In addition to the antibody properties, it appears that the high concentration of platelets in thrombus, i.e., the high antigen "thrombus-to-blood ratio", defined as "receptor density", was also a major factor for the high-contrast images obtained within a short time. It was further found that this method enables imaging of fresh, one-day-old thrombi, but was negative in cases of thrombi two days of age. Peters et al. (*2*) using a similar antiplatelet antibody (P256) labeled with ^{111}In were able to image venous thrombi in humans. Subsequently, a second monoclonal antibody reacting with platelets, 5OH.19 (*3*) was evaluated. This preparation consisted of a mixture of fragments (F(ab')-85%; F(ab')$_2$-15%), rather than the whole antibody, and was supplied in kit form for one-step ^{99m}Tc labeling. The preparation showed high immunoreactivity to platelets and clots, but no binding to other blood elements. Blood half-disappearance time was 3–6 min with 18%–24% of the injected activity appearing in the urine in the first 3 hr after injection. Imaging results were similar to those obtained with 7E3, i.e., experimental thrombi in veins and arteries in the neck and extremities (carotid, femoral arteries, jugular vein, and femoral vein) as well as in right ventricle and in segmental pulmonary arteries were imaged 60–90 min postinjection. The thrombus/

blood ratio measured ex vivo was found to be 15 on average. Spontaneous thrombi which formed on indwelling catheters also appeared as "hot spots," and sites of intimal damage without visible thrombus formation were also positive. In addition, thrombosed bowel could also be imaged by using ^{99m}Tc-50H.19 (*4*). A further refinement of the antiplatelet antibody method was recently proposed by Palabrica and colleagues (*5*) who demonstrated thrombus imaging with antibodies against activated platelets in a primate model. A monoclonal antibody, KC4, reacting selectively with human activated platelets could be used only for in vitro experiments at this stage. A similar polyclonal antibody, anti-PADGEM (platelet activation-dependent granule-external membrane protein) was used in primates with experimental thrombi. Using what seems to be the best animal model (primates) of DVT and experimental techniques, they have shown KC4 binding of 90% ± 10% to a dacron graft model compared to 70% ± 12% for the polyclonal anti-PADGEM antibody. Ex vivo thrombi on the dacron graft could be visualized 10 min after ^{123}I-labeled antibody injection with optimum images obtained at 30 min. After the injection of 260 μCi ^{123}I-anti-PADGEM (100 μg protein) to baboons, experimental DVT could be imaged 15 min postinjection, with an optimal scanning time of 30–60 min. Anti-PADGEM had a half-disappearance time of 6 min. In yet another publication, it was shown that the antiplatelet antibody technique may also be of value for monitoring thrombus dissolution caused by streptokinase (SK), urokinase (UK), or recombinant tissue plasminogen activator (rt-PA) (*6,7*). Standardized clots incubated with ^{99m}Tc-50H.19 were washed, counted, and incubated with graded concentrations of SK, UK, or rt-PA for various time intervals. Clot lysis was followed to almost completion. After repeated washings, it was found that the decrease in dry weight of clot as a result of thrombolysis paralleled the decrease in ^{99m}Tc counts in residual clot. This in vitro correlation indicates that it may be possible to monitor thrombus dissolution by scintiimaging of the disappearance of the "hot spot" over a clot caused by thrombolytic agents.

THE ANTIFIBRIN ANTIBODY APPROACH

The first monoclonal antifibrin antibody 59D8, developed by Hui et al. (*8*) against a synthetic fibrin-like peptide of the beta chain, was followed by T2G1s developed by Kudryk (*9*), both antibodies probably sharing the same epitope on the fibrin molecule (N$\bar{H}$ terminus of beta chain of fibrin). This epitope becomes exposed only after the action of thrombin on fibrinogen. A third antibody investigated recently in humans and termed C22A is presented as identical to 59D8 (*10*). Although other antifibrin antibodies have been described (*11–14*), 59D8 and T2G1s has been studied most extensively. These whole antibodies, and various monomeric and dimeric fragment preparations (Fab, Fab′ and F(ab′)2), have been investigated in the laboratory and in clinical trials using ^{131}I, ^{111}In and recently ^{99m}Tc as the radiolabel (*15–22*). An interesting development in antifibrin antibody imaging has been the refinement described in this issue of the *Journal* by Rosebrough and colleagues (*23*). As mentioned earlier, the epitope for TG21s and 59D8 binding becomes exposed as a result of the digestive action of thrombi on fibrinogen. However, this site is cleaved off early during fibrinolysis, thus becoming unavailable for further antibody binding. Sites already occupied by antibody are probably not affected by fibrinolysis as evidenced by the concentration of labeled antibody bound to thrombus which remains constant. Thus, GC4 described by Rosebrough enables better imaging of older thrombi.

The feasibility of imaging thrombus with T2G1s (*15*) and 59D8 (*17*) labeled with radioiodine and ^{111}In was clearly demonstrated in experimental animals using thrombogenic coils. It appears that T2G1s enables visualization of thrombi that are several days old (*16*). Since the main focus of research was directed to the diagnosis of DVT, it was fully justified to search for a method of detecting thrombi that are several days old, because most patients usually present with this condition after many days of pain, swelling, or other symptoms of DVT.

The new antibody GC4, reacts with an epitope on the D fragment of fibrin which becomes exposed after plasmin digestion, i.e., later in the natural history of thrombus aging (*23*). In vitro, GC4 binds with the fibrin monomer, with cross-linked fibrin and with plasmin-digested fibrinogen. GC4 was compared to T2G1s, each antibody labeled with a different isotope, so as to enable simultaneous evaluation of the two antibodies. The antibody protein was kept constant at ~50 μg and the radiolabels were 250 μCi ^{131}I or 50 μCi ^{125}I or 300 μCi ^{111}In. Some of the experimental animals were heparinized 3 hr before antibody injection. Thrombi were from 3 hr to 3 days old. It was shown that GC4 binds to fibrin on specific and saturable binding sites and that TG21s does not compete for the GC4 binding sites, thus proving that these are clearly two different antibodies. It was also shown that 3-hr old thrombi could be imaged with ^{131}I-GC4 only 24 hr after antibody injection. During this time the thrombus matures, more antibody binds to the thrombus, and background activity decreases. A surprising and intriguing finding is that heparin enhances GC4 uptake by thrombus and earlier imaging is possible. The same finding was observed when using ^{111}In-GC4 or ^{131}I-GC4. As the thrombus ages, the GC4/T2G1s ratio increases and in heparinized animals GC4 concentration in clot was 1.9 times that of T2G1s. The authors present the view that heparin

prevents "blanketing" or "covering" of the GC4 epitope by new thrombus growth. Thus, GC4 binding sites remain exposed allowing more antibody to bind. Whether this phenomenon of heparin augmentation of GC4 uptake will also be found in clinical situations is not clear.

Thrombogenic coils, as used in this and other studies as experimental models of DVT, may not undergo the same aging processes as spontaneous venous thrombi. It may well be that the thrombogenic effect of the coils persists until there is complete and dense epithelization, and thus sites of new and old thrombi may probably be present, simultaneously. The clot/blood ratios of GC4 increase with the age of the clot from 9 at 3 hr to 12 at 24 hr and in the presence of heparin to 24, while TG21s ratios are 9 at 3 hr and 5 at 24 hr. The blood disappearance of GC4 is faster as compared to TG21s, further contributing to better thrombus/blood ratio. Following the results in animals that demonstrated the efficacy and safety of whole antibody and fragment preparations, human studies were first reported in 1988 (*20–22*). These three reports clearly indicate that "hot spots" over the thrombus area could be consistently visualized. The doses used in human were in the range of 2 mCi ^{111}In with 0.5 mg antibody protein (*20*). These resulted in radiation doses of 4.0 rad/mCi in kidneys and 0.26 rad/mCi to whole body (*10*) which are within the acceptable range.

ANTIPLATELET OR ANTIFIBRIN ANTIBODIES—WHICH "MAGIC BULLET" IS BETTER?

It is clear from the aforementioned evidence that both antiplatelet and antifibrin antibodies are suitable for thrombus imaging. Both preparations seem to have advantages when given as Fab fragments, without the Fc portion. Monomers may be better than the dimers, because of the lower molecular weight which may possibly improve antibody penetration into thrombus. The presence of the Fc in the fragment preparation reduces blood clearance and increases the likelihood of immune reactions. It is also beyond discussion that ^{99m}Tc is the ideal radiolabel for these applications. Although the gamma emissions of ^{123}I are close to those emitted by ^{99m}Tc, in vivo dehalogenation, the high cost of purer ^{123}I, and the unavailability of an antibody kit for iodination are all in favor of a ^{99m}Tc label. Indium-111 is also not as good as ^{99m}Tc. The administered doses are smaller and transchelation is a serious problem.

Antifibrin antibodies were evaluated much more extensively as compared to antiplatelet antibodies. Human studies indicate that these preparations are safe and effective and further studies will show the clinical advantages of these methods.

The direct comparison of antiplatelet and antifibrin antibodies is limited to a single abstract publication (*24*) showing a slightly higher uptake of the antiplatelet antibody in thrombus as compared to the antifibrin. Imaging thrombi in the trunk with antifibrin antibody as also been described, although the results are yet inconclusive (*25,26*), however most reports focus on the diagnosis of DVT.

It may well be that antifibrin antibodies are in general more advantageous for the detection of DVT. GC4 has definitely increased the detection span of the procedure for even older thrombi.

Antiplatelet antibodies on the other hand, seem to hold great promise. The possibility of detection of venous as well as of arterial thrombi in the extremities and in high blood-pool areas within the time-frame of the "lytic window" (*27*), that is, imaging that can diagnose active thrombi still amenable to thrombolytic therapy, as opposed to older thrombi, now seems possible (*28*). The possibility of scintigraphic monitoring of thrombolysis is no less exciting.

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