Editorial Comment

Imaging Arterial Thrombi
An Elusive Goal

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The role of thrombotic processes in the conversion of a quiescent atherosclerotic plaque into a clinically active atherothrombotic lesion has been well established over the past decade. In coronary, cerebral, and peripheral arterial beds, atherothrombotic events underlie acute clinical vascular syndromes ranging from unstable angina and myocardial infarction to transient ischemic attack and stroke.

In the coronary circulation in particular, recognition of the role of thrombosis in acute myocardial infarction is largely responsible for the recent development and clinical application of thrombolytic therapy. Because invasive angiographic assessment is not logistically feasible or ethically acceptable before the initiation of thrombolytic therapy in most patients with evolving infarction, the diagnosis is made without firm evidence for the presence of an acute thrombotic coronary process, let alone unequivocal identification of the involved vessel: The localization of a “culprit” lesion in acute infarction rests largely on inferential electrocardiographic, echocardiographic, or scintigraphic data.

The lack of a simple, relatively noninvasive method for identifying an acute atherothrombotic stenosis or occlusion is a notable deficiency in current cardiovascular care. Inaccurate diagnosis, ineffective therapeutic response, and reocclusion or restenosis after initially successful therapy are all potential pitfalls of current approaches to patients with suspected acute occlusive arterial syndromes; improvement in diagnosis and management would be facilitated by an efficient and specific method for defining noninvasively and unequivocally the status of the culprit vascular lesion.

The majority of studies and methods developed to image thrombi involve the use of radionuclide imaging agents. The earliest successful scintigraphic approach was that using [125I]-fibrinogen in the diagnosis of deep vein thrombosis. This method, still currently in use, requires an actively growing thrombus and several days of imaging for optimal diagnostic sensitivity. However, labeling the large circulating fibrinogen pool results in a low thrombus-to-blood activity ratio; this requires that imaging be deferred until adequate clearance of circulating label has occurred to provide target-to-background activity ratios sufficient for thrombus detection.

The experience with [125I]-fibrinogen in deep vein thrombosis highlights the principal problems with which one is confronted in scintigraphic imaging of thrombus. First and foremost, one must label some component of the thrombus relatively selectively; ideally, that component should not be present in the circulating blood pool. Second, the element of the thrombus that is labeled must be in sufficiently great local concentration or be labeled at high enough specific radioactivity to facilitate detection by external imaging.

Over the past 20 years, a number of approaches have been used to minimize these problems and have met with variable degrees of success. Three principal elements of the thrombus have been chosen as targets for labeling: fibrin, platelets, and fibrinolytic molecules. Antibodies targeted to epitopes on these components or directly labeled components have been used. After the experience with radiolabeled fibrinogen, the field advanced only with the development of monoclonal antibody technology for the preparation of antibodies that distinguish epitopes on fibrin from those on fibrinogen. Antibodies to platelets incorporated in thrombi have also been used, including an antibody to the fibrinogen receptor glycoprotein IIb/IIIa, one to an antigen expressed only on activated platelets (PADGEM), and one to radiolabeled autologous platelets directly. The first two methods suffer primarily from being able to detect only fresh thrombi, whereas in the last-named method, radiolabeled erythrocytes must be used concomitantly to reduce the blood pool background. Inasmuch as plasminogen binds to fibrin through its kringle domains, one group has attempted to use radiolabeled plasminogen as an imaging agent.

Others have used radiolabeled streptocinase and urokinase, arguing that their affinity for plasminogen bound to the thrombus will facilitate uptake of the labeled molecule. These studies, again,

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have suffered primarily from lack of adequate sensitivity to detect small thrombi.

Alternative approaches for the detection by radiouclide scintigraphy of coronary occlusion in particular involves the recent application of perfusion imaging agents such as $[^{99m}Tc]$-sestamibi or the more short-lived compound $[^{99m}Tc]$-teboroxime. These methods suffer primarily from the lack of direct imaging of thrombi and the requirement for poor collateralization to ensure sufficiently reduced perfusion for detection; they do, however, provide information about the functional importance of an occlusive process.

In this issue of Circulation, two groups present work on thrombus imaging with two newly developed methodologies. Cerqueira and colleagues used a murine monoclonal antibody, T2G1s, which recognizes fibrin’s aminoterminal $\beta$-chain heptapeptide selectively. They improved on earlier studies by using a similar monoclonal, 59D8, by 1) producing Fab fragments to enhance clearance and thereby reduce background signal and 2) labeling with $[^{99m}Tc]$ rather than $[^{111}In]$ or $[^{131}I]$ to exploit the high photon flux of the former agent for Anger camera imaging in humans. A clot-to-blood ratio of 4.24 and the ability to image coronary thrombi 2 hours after injection of label represent a significant improvement over prior methods using polyclonal antifibrinogen antibodies or monoclonals labeled with radionuclides different from $[^{99m}Tc]$. Several shortcomings, however, limit the broad applicability of this methodology to clinical situations. Only the ends of occlusive thrombi were imaged, and patchy uptake was noted in partially occluding thrombi. The clots formed were relatively platelet poor and, therefore, unlikely to be characteristic of arterial thrombi formed under pathophysiological circumstances; enriching the clots with platelets would likely lead to even lower uptake of antibody, given the relative depletion of fibrin produced by the increased platelet mass. Finally, poor image quality was noted over the coronaries in particular, very likely as a consequence of limited resolution capability of the method for a clot of this size.

Attempts to define thrombus require attention to both spatial and contrast resolution. For example, conventional Anger camera imaging using $[^{99m}Tc]$-labeled agents under the best experimental conditions optimally achieves spatial resolution on the order of 1 cm. This is clearly inadequate for imaging most coronary thrombi but might be acceptable for thrombus detection in larger-sized vessels. One approach to enhancing spatial resolution involves the use of alternative collimation systems, for example, a 1–2-mm pinhole aperture. However, because spatial resolution is inversely related to collimator sensitivity, this substitution would obligate enhanced activity at the site of thrombus detection to maintain adequate contrast resolution.

In the second study, Ord and colleagues used tissue-type plasminogen activator (t-PA) inactivated with D-phenyl-L-prolyl-L-arginyl-chloromethyl ketone (PPACK) and conjugated to a radioiodinated residualizing label, dilactil tyramine (DLT) ($[^{131}I]$-DLT-PPACK-t-PA) to image arterial clots. When administered concomitantly during clot formation, $[^{131}I]$-DLT-PPACK-t-PA manifested strikingly elevated clot-to-blood ratios in the carotid, pulmonary, and coronary vessels of 37, 42, and 137, respectively. Administration 10 minutes to 1 hour after clot formation, however, led to reduced but respectable ratios of 28, 8, and 18, respectively, for the carotid, pulmonary, and femoral vessels. No ratio was reported with administration of label after clot formation for coronary vessels. Single-photon emission computed tomography (SPECT) imaging of carotid clots was successful with infusion of label during clot formation and of femoral clots with infusion of label after clot formation. The success of this method for imaging clots in relatively small vessels (despite the use of $[^{131}I]$) likely accounted for the high clot-to-blood ratios achieved with the highly specific and clot-avid t-PA derivative. That the SPECT technique enhances the sensitivity of detection follows from the fact that SPECT acquisition enhances contrast resolution (i.e., it improves definition of target versus background activity). However, this improvement in contrast resolution occurs at the expense of spatial resolution and, therefore, may not be as useful when applied to imaging thrombi in the coronary circulation.

This innovative approach is biologically sound: blocking the active site of t-PA with PPACK prevents dissolution of the clot by the labeled plasminogen activator which, while therapeutically beneficial, would limit its diagnostic potential by dissolving the fibrin substrate to which it must bind for imaging. Data are also provided that indicate the lack of impairment of fibrinolysis by relatively low absolute concentrations of this inactivated and potentially competitive derivative of t-PA. The use of the residualizing label DLT facilitates exclusion of potential metabolic, competitive proteolytic degradation fragments of $[^{131}I]$-t-PA from the vascular compartment that would decrease the signal-to-noise (i.e., clot-to-blood) ratio. Several important shortcomings are apparent with the use of this agent. Less $[^{131}I]$-DLT-PPACK-t-PA bound to clots with increasing age, likely as a consequence of diffusion limitation into an increasing crosslinked fibrin matrix. Clot-to-blood ratios were greatest when imaging agent was added during clot formation. The specificity of the agent for thrombi may be less than ideal, given the propensity of t-PA to bind to specific surface receptors on the endothelium.

Although both of these studies have significant shortcomings, they represent considerable improvements over prior attempts at achieving the elusive goal of imaging relatively small arterial thrombi. For such an agent to be clinically useful, it must not only be thrombus specific and of high enough specific radioactivity for technically acceptable images but
must also be taken up by thrombi rich in platelets or fibrin, by crosslinked aging thrombi as well as fresh thrombi, and throughout the anatomic extent of the thrombus. Neither these two agents nor any agents studied previously meet these important criteria sufficiently to support broad clinical application.

Given these requirements, are there alternative strategies that one might consider in designing future imaging agents for arterial thrombi? Simple approaches that might further improve on existing methods include the use of \(^{99m}\text{Tc}\) as the radionuclide with which DLT-PPACK-t-PA is labeled and the use of radiolabeled monoclonal antibodies that recognize crosslinked aged thrombi better than nascent thrombi, such as the murine monoclonal GC4.\(^{24}\) A combination of T2G1s and GC4 may also be considered and used to label thrombi of varying ages. Molecular biological approaches may also be used to produce molecular fragments tailored to recognize thrombus components selectively, such as the first kringle domain of plasminogen, a relatively small and, therefore, theoretically more thrombus-penetrable molecular structure that binds to fibrin with high affinity.\(^{25}\) Radiolabeled recombinant thrombin inhibitors such as desulfato-hirudin\(^{26}\) or hirugen\(^{27}\) may also be used both to image thrombi and limit their growth. That clot-bound thrombin is less well inhibited by standard anticoagulant heparin than by these derivative leech proteins\(^{28}\) and that circulating free thrombin exists at a concentration significantly less than that bound to clot support both the diagnostic and potentially therapeutic strengths of this type of approach. Combinations of antplatelet and antifibrin antibodies such as radiolabeled anti-PADGEM and radiolabeled T2G1s may also be used to overcome the potential limitations that relative platelet or fibrin enrichment of a given thrombus produce when imaging is attempted with antifibrin or antplatelet antibodies alone, respectively. Radiolabeled antibodies may be used to increase the specific labeling of, for example, a relatively fibrin-rich thrombus imaged initially with a murine monoclonal anti-PADGEM antibody and also to increase the contrast resolution when improved collimator technologies are used to increase spatial resolution.

These and other similar radionuclear approaches hold promise for future developments in this difficult diagnostic area; however, it is important to point out that given current technological limitations of radionuclide imaging, none of the methodologies that have been developed thus far or discussed in this editorial is likely to meet all of the idealized requirements presented above. As a promising alternative to these methods, improved resolution of medium-sized vessels and, eventually, of moving coronary vessels specifically by ultrafast computed tomography or vascular magnetic resonance imaging techniques may provide the ultimate solution to this difficult but very important problem in diagnostic cardiovascular medicine.

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