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Imaging Vulnerable Plaque
A radionuclide approach

H. William Strauss, M.D.[1]
Section of Nuclear Medicine, Memorial Sloan Kettering Cancer Center,
New York, NY 10021 United States
E-mail: straussh@mskcc.org

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1. Introduction

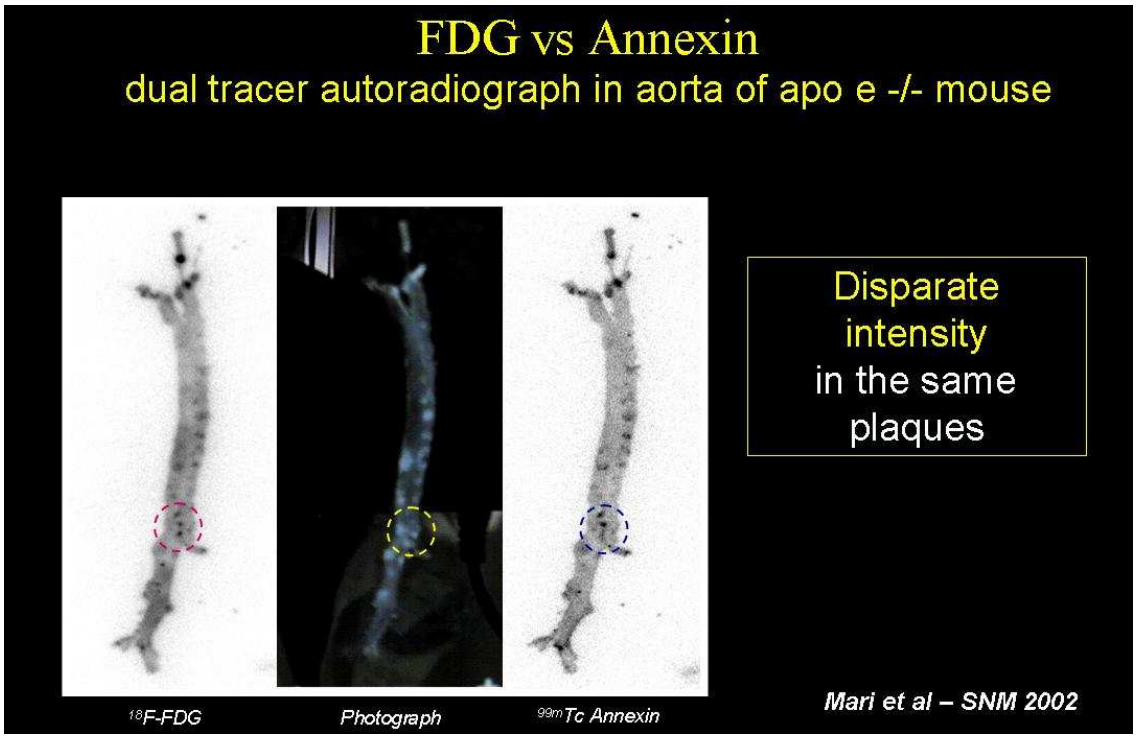
Atherosclerosis is a systemic immune inflammatory disease with cycles of progression followed by intervals of stabilization.[i] These cycles occur multiple times over decades. In the later stages there are changes in the plaque cap leading to formation of thrombus. These thrombi are usually non-occlusive, and therefore clinically silent. There are three different processes involving the cap of the plaque that lead to thrombus formation. The most common is a loss of integrity of the cap of the plaque, bringing the thrombogenic plaque contents into contact with blood. A second mechanism is termed 'erosion.' This process is the loss of the endothelial cells on the cap of the plaque, exposing blood to the thrombogenic glycoproteins and collagen beneath these cells, resulting in formation of thrombus. Histologically, the cap of the plaque is still intact. A third phenomena, which is less common, is the formation of calcium nodules in the cap of the plaque (likely secondary to the apoptotic death of macrophages in the plaque). The nodules lead to marked local turbulence, and activation of clotting factors. Regardless of the cause of thrombus, the majority of clots undergo resorption and endogenous thombolysis, with stabilization of the lesion. A small fraction of thrombi cause an occlusive thrombus, leading to an acute vascular event.

Although the etiology of plaque rupture is incompletely understood, the presence of glycophorin in the plaque (from extravasated hemoglobin) indicates that the loss of integrity of vasa vasorum from intraplaque hemorrhage is a common event.[ii] Extravasated red cells add to plaque burden. The extravasated red cell loses its integrity, spilling hemoglobin into the lesion, and leaving the cell membrane, with its high concentration of lipids and cholesterol, to disintegrate. The lipid in the cell membrane contributes to the formation of cholesterol crystals in the lesion. The free hemoglobin (as well as the iron that may be released from the hemoglobin) creates an oxidizing environment, particularly irritating to macrophages, which adds to macrophage activation. The elimination of extravasated hemoglobin depends on hemoglobin combining with the plasma protein haptoglobin, and subsequent ingestion/digestion of the complex by the macrophage. Increasing macrophage metabolism requires increased delivery of oxygen and metabolic substrates into the lesion, resulting in angiogenesis stimulating further growth of vasa vasorum, which raises the likelihood of further plaque instability. Each episode of extravasation increases intraplaque pressure (at least transiently), increasing the likelihood of plaque rupture. Stabilization of the lesion, on the other hand, is associated with fibrosis/calcification, decreased lipid and inflammatory cell content and a marked decrease in vasa vasorum.

Non-invasive imaging techniques may identify atheroma, but do not characterize the lesion. For example, typical cardiac stress testing, which increases regional flow with exercise, dobutamine or vasodilators, defines areas of limited flow reserve.[iii] Similarly, CT angiography identifies narrowings. In many cases, the information gleaned from these studies is sufficient to make a clinical decision. In the symptomatic patient with severely limited flow reserve, revascularization is indicated and no further lesion characterization is necessary. On the other hand, in the patient with atypical symptoms or minimally impaired flow reserve, plaque

characterization may be helpful. Similarly in the asymptomatic patient with risk factors, mapping the distribution of vulnerable plaques may be clinically useful. If the atheroma is in a phase of progression, lipid lowering coupled with diet, exercise and lifestyle changes may be most helpful.

To characterize the lesion with radionuclide techniques the tracer must localize in the lesion and clear from the blood and normal tissues. To accomplish this the tracer must be delivered to the site(s) where it will concentrate and be retained long enough to permit imaging. To detect lesions undergoing progression, we can consider imaging with markers of lipid deposition (radiolabeled low-density-lipoprotein or oxidized lipoprotein), inflammatory cell attraction (increased expression of integrins and chemoattractant factors), inflammatory cell metabolism (macrophages catabolize exogenous glucose, which is obtained from plasma/extracellular fluid), proliferation of vasa vasorum (which may lead to intraplaque hemorrhage) and in the late phases of the severely inflamed lesion, recruitment of lymphocytes and granulocytes. In addition to their metabolic activity, foam cells undergo apoptosis due to the toxicity of oxidized lipoproteins in the lesion. In the process of apoptosis, the cells express phosphatidylserine on the external leaflet of the cell membrane, which can be detected with radiolabeled Annexin V. Figure 1 (spectrum of metabolic changes in atheroma) summarizes some of the potential imaging agents [legend the spectrum of metabolic changes in atheroma are summarized in the cartoon.



Imaging vulnerable plaque provides a total body survey to detect the site(s) of atheroma and potentially defines the likelihood of progression at each location. The radiolabeled glucose analog, FDG, localizes at sites of increased macrophage activity in the vasculature (reference wahl, alavi and Dunphy). At the plaque evolves from stable to vulnerable more lipid is deposited leading to upregulation of chemotactic peptide expression, increased metabolic

activity of inflammatory cells in the lesion and apoptosis of some cellular elements. These changes can be identified with several radiolabeled substances as summarized in Table 1.

The expression of each of these factors depends on the stage of the plaque. Figure 2 is a dual tracer autoradiograph of the aorta from an apo-e -/- mouse. The animal was injected with both FDG and Tc-99m annexin V. About one hour after injection the aorta was harvested, photographed (center panel) the FDG autoradiograph was recorded (left panel), followed about 8 hours later by the Tc-99m autoradiograph (right panel). Although the distribution of the two tracers is similar, it is not identical. Some lesions have more FDG, others, more annexin. This disparity suggests that increased metabolism of cells within plaques occurs at different time and to a different degree than apoptosis. It is not clear which of the available agents will be best to identify plaques at each phase of their cycle.

TABLE 1
The following is a partial list of agents that have been suggested for imaging atheroma:

- Lipoproteins
  - LDL – labeled with 125I, 99mTc, 111In
  - Oxidized LDL – labeled with 99mTc
- Change of phenotype of vascular smooth muscle cells from contractile to proliferating
  - Antibody recognizing a unique epitope on proliferating smooth muscle cells
    - (Z2D3) labeled with 99mTc
- Inflammation in atheroma
  - Glucose utilization of inflammatory cells
    - 18FDG
  - Apoptosis (primarily caused by toxicity of oxidizied LDL in macrophages)
    - 99mTc-Annexin
  - Increased expression of integrins
    - RGD peptides
  - Increased expression of chemotactic factors
    - 99mTc-MCP-1
  - Increased expression of folate receptors on activated macrophages
    - 99mTc-Folate
- Fibronectin – modified with splice variant ‘extra domain B’

From a clinical perspective, it may be best to identify lesions that are accruing lipid, but do not yet have severe inflammation, since changes in diet and lipid lowering medication is likely to be successful. On the other hand, it may be better to use overall plaque metabolism as the major marker, since inflamed plaques are associated with rupture. FDG will work as a marker for this approach, since macrophages use exogenous glucose as their major substrate. This approach has confirmed the findings at pathology and by intravascular ultrasound (in the coronary arteries)[iv] that multiple lesions are inflamed at the same time. These lesions exist in multiple sites, including the cerebral and coronary circulations. The multiplicity of lesions, and the low incidence of clinical events as a result of these lesions, makes it very likely that the imaging examination will have a high sensitivity but a very low specificity. As a result, an imaging procedure identifying inflamed sites in the vasculature will require quantitation to be clinically useful.

Major challenges to imaging atheroma are spatial resolution and motion. Autoradiography of tissue specimens demonstrates adequate contrast between lesion and normal vessel. In vivo,

however, there are several problems. First, residual tracer in the blood and surrounding tissues decreases contrast. Second, pulsatile motion of the artery blurs the data. Third, the size of an average atheroma, <10mm^3, is considerably smaller than the resolution of the imaging device: in vivo resolution of PET is ~ 256mm^3 and ~ 1000mm^3 for SPECT. Detection of a lesion that is smaller than the resolution of the imaging device requires a marked increase in the ratio of activity in the lesion compared to that in surrounding tissue. To detect atheroma in a coronary artery, the ratio between activity in the target and that in the background will need to increase by a factor of ~ 25 for PET and ~ 100 for SPECT, compared to that required to detect a lesion that is 2x larger than the resolution of the instrument.

Each of these three factors can be addressed. Assuming ^{18}FDG as the tracer, the following are several steps that may contribute to recording a diagnostically useful image:

1. Increasing the ratio of tracer in the target compared to that in the background.
    a. Activity in the background can be minimized by recording images 3 hours after tracer injection.
    b. Preparing the patient with a high fat diet, which will decrease insulin secretion, will decrease uptake in normal myocardium, and likely also in normal tissue.
2. Pulsatile motion of the vessel can be minimized by:
    a. Recording vessel uptake into multiple frames using the patient's R wave for synchronization
    b. Respiratory gating will also be necessary, since the heart moves during tidal respiration.
3. Using data from CT (especially contrast CT angiography), will allow application of resolution recovery algorithms, for both detection and quantitation of lesion uptake.

2. Conclusion

Inflammation is an integral component of atheroma. There are a number of radiolabeled markers that can be used to detect inflammation, which may be useful for imaging atheroma. The glucose analog FDG localizes in atheroma, and may be suitable to characterize the severity of inflammation Iesions. A number of daunting technical hurdles remain, before FDG vascular imaging can be applied to routine clinical care. However, the preliminary results appear very promising.

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