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Finding Calcium in Non-calcified Lesions:

^{18}F-Fluoride Offers Insights Into Atheroma Evolution

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'*It is not what you look at that matters, it is what you see.' Henry David Thoreau*

Intimal arterial calcification is "a pervasive and likely inevitable program that is intimately entwined with aging, atherosclerosis and cardiovascular disease.[1]" Since 1990 vascular calcification has been quantitated[2] and correlated with the likelihood of cardiovascular events[3]. The clinical significance of intimal arterial calcification, however, remains controversial. Shaw et al[4] reviewed the issues in a recent editorial entitled "The never ending story of coronary calcium: is it predictive, punitive or protective?" To understand why this controversy exists it may be helpful to evaluate the pathophysiology of atheroma.

Calcification of atheroma occurs in inflamed lesions. Lesions are inflamed because LDL cholesterol is trapped in the subintimal space, irritating the overlying endothelium, leading to the production of chemotactic factors. The chemotactic factors attract mononuclear cells to the site[5,6]. Mononuclear cells transform to tissue macrophages, enter the lesion and phagocytize the LDL cholesterol complex. The LDL cholesterol complex is difficult to catabolize, and in the process of breaking down the protein-lipid complex, oxidized LDL is formed. Oxidized LDL is toxic to the macrophage, resulting in apoptosis or necrosis of the cell. Loss of integrity of the cell membrane of necrotic cells results in release of oxidized LDL as well as numerous proteases into the lesion, increasing the inflammation. The toxic environment causes apoptosis of some adjacent cells, such as smooth muscle cells, causing release of matrix vesicles[7], further increasing inflammation. The usual 'clean-up and recycling' of elements from these dead cells by efferocytosis is impaired[8] by a combination of the age of the patient and severity of inflammation[9].The persistence of the toxic lesion attracts additional monocytes, mast cells, and lymphocytes which produce factors, such as bone morphogenetic protein-2, that lead to the formation of microcalcifications[10].

Microcalcifications are seen in lesions with pathological intimal thickening, and appear as microscopic foci, typically >0.5um and < 15 um at histopathology[7]. These lesions are too small to be resolved by conventional CT, which has a spatial resolution of ~ 6 line pairs/cm in a high contrast phantom[11], and a resolution of approximately 5 mm for low contrast objects[12]. As lesions progress through multiple cycles of inflammation and healing, the layers of calcification progresses from microcalcifications to sheets of dense calcium, which are large and dense enough to be seen on CT.

In addition to calcification, another characteristic of atheroma is increased angiogenesis. Increased vascularity of the lesion enhances delivery of circulating substances to the lesion. Even large molecules,

such as radioiodinated autologous low density lipoprotein[13], molecular weight ~ $3x10^6$, localize in carotid atheroma within hours of intravenous injection[14], likely due to mixing and retention in the large intralesional pool of LDL. Given the localization of large molecules, it is not surprising that small particles, such as radiofluoride ions (fluorine-18 as fluoride ion) also localize in these lesions[15]. It is likely that the mechanism of fluoride localization is due to adsorption of the ion on the small dystrophic particles of calcification (with a large surface area).

Identifying these microcalcifications is important, because a finite element analysis of 35,000 microcalcifications in lesions from 22 patients demonstrated that, depending on the size and distance between particle pairs and the orientation of the particles with reference to the tensile axis of the cap, the local tissue stress could increase by a factor of 5[16], raising the likelihood of cap rupture.

In this issue of the Journal, Fiz et al[17] describe the relationship of ionic fluoride-18 localization in the infra-renal abdominal aorta to the distribution of CT visible calcification on PET-CT images. The authors observed an average of 6.2 sites of arterial calcification/patient (providing 397 foci of arterial calcification for analysis). There was an ***inverse*** correlation between fluoride localization and Hounsfield unit plaque density. In fact, the authors found fluoride hot spots at sites ***without*** visible calcification in 86% of patients. Fluoride intensity at these sites was higher than that observed at sites with calcification on CT.

The data reported by Fiz[17] suggest that fluorine 18 localizes at sites of calcification that is invisible at current clinical CT resolution. Although many of these lesions may not be at imminent risk of rupture, due the distance between foci or their orientation with reference to the tensile strength of the plaque, it seems that these lesions are the seat of intense activity with abundance of inflammation, cytokine excess and ongoing cellular damage. Such active lesions may result in rapid necrotic core expansion and plaque progression, which happens to be the strong predictor of eventful plaques[18].

The current investigation is an exciting hypothesis generating study and we need prospective outcomes data to establish the value of serial NaF vascular imaging. NaF imaging might evolve to supplement the overall prognostic importance of the Agatston score by offering lesion-specific prognostic information.

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