THE IMPACT OF CALCIUM ON ARFI IMAGING OF ATHEROSCLEROTIC PLAQUES

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Submitted for publication in final form: October 30, 2008.

Abstract: Cardiovascular risk stratification could be improved by properly delineating the position of calcium deposits relative to other plaque structures in atherosclerosis imaging. ARFI ultrasound has been demonstrated to resolve plaque collagen and elastin composition, in vivo and ex vivo, but its ability to localize calcium deposits has not been thoroughly explored. We here evaluate the impact of calcium deposits on ARFI imaging of atherosclerotic plaques in a familial hypercholesterolemic pig model.

I. Introduction: Although coronary calcium deposits are well-established predictors of cardiac morbidity and mortality, the overall role of calcium deposits in atherosclerosis and their contribution to plaque stability remains controversial [1]. Recent evidence based on mathematical modeling and postmortem studies supports that when positioned in the plaque shoulder, calcium deposits stabilize plaques. However, when positioned in the fibrous cap, calcium deposits substantially compromise plaque stability [1-3]. Even 10-20 µm microcalcifications in thin fibrous caps have been shown to significantly increase plaque rupture potential, while calcium deposits positioned in lipid cores or far from the arterial lumen are reported to have little impact on overall plaque stability [3]. These data suggest that improvements in cardiovascular risk stratification may be gained by properly identifying the position of calcium deposits relative to other atherosclerotic plaque structures.

Atherosclerotic plaque structure and composition can be assessed noninvasively by Acoustic Radiation Force Impulse (ARFI) ultrasound. We have previously demonstrated ARFI’s potential for in vivo delineation of plaque collagen and elastin content and identification of fibrous cap and lipid core structures in a familial hypercholesterolemic pig model [4]. However, we have not evaluated ARFI’s utility for localizing calcium deposits, nor have we investigated how calcium deposits impact ARFI’s ability to resolve other plaque structures. Therefore, we now explore the impact of calcium deposits on ARFI imaging outcomes in a familial hypercholesterolemic pig model, ex vivo and in vivo, with immunohistochemical validation.

II. Methods: ARFI imaging was performed using a Siemens SONOLINE Antares™ imaging system equipped for research purposes and VF7-3 linear array transducer (Siemens Medical Solutions USA, Inc., Ultrasound Division). ARFI excitation impulses were 300 cycles (70 µs) in duration with a center frequency of 4.21 MHz and an F#/1.5 focal configuration. Tracking pulses were 2 cycles in duration with a center frequency of 6.15 MHz at an 11 kHz PRF. Tracking ensembles of ~6 ms in length were acquired in 15 lateral positions, with 4:1 parallel receive, for a total of 60 lateral tracking positions spaced evenly across a 2 cm lateral FOV. ARFI excitation and tracking occurred in the same lateral position. Axial tissue displacements were measured by one-dimensional cross-correlation. Physiological motion was rejected using a long wave assuming polynomial regression filter [5], and signal from the arterial lumen was masked based on cross-correlation variance and signal amplitude.

In vivo imaging was performed by a sonographer trained in peripheral vascular ultrasound in 4 familial hypercholesterolemic pigs. The pigs were female and aged 7 yr 4 mo, 7 yr 2 mo, 5 yr 9 mo, and 5 yr 1 mo. The animals were sedated and positioned in lateral recumbency while data was collected from the left and right iliac arteries. A carbon particle solution (undiluted Carbon Black Dispersate No. 8; Eberhard Faber, Bedminster, New Jersey) was injected into the arterial adventitia and surrounding soft tissue to mark the location of imaging, and morphological features were noted to ensure proper spatial registration of excised arterial specimens to imaging data [6]. After in vivo imaging was completed, the vessels were harvested by a veterinary surgeon at necropsy, which occurred within 48 hours of imaging. The freshly excised
vessels were tethered to a custom hydrostatic pressure apparatus to maintain luminal pressure during ARFI and SWEI imaging. Upon completion of ex vivo imaging, the arteries were sectioned and stained with hematoxylin and eosin (H&E) for baseline, Verhoeff van Gieson (VVG) for elastin, Masson’s trichrome (MT) for collagen, and von Kossa (VK) for calcium. All procedures were approved by the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill.

III. Results and Discussion: Figure 1 illustrates the immunohistochemical results obtained from an iliac plaque in the 5 yr 9 mo old pig. In VVG (panel a, showing elastin black) and MT (panel b, showing collagen blue) stains, a thin fibrous cap (region 1) and a deposition of foam cells (region 2) are evident. The VVG stain shows elastin deposition in the fibrous cap (region 1, arrows), in region 3 (arrows), and in the bottom, medial portion of the arterial wall (arrows). The MT stain shows collagen deposition in a line in the plaque shoulder (region 4, arrows) and across a large area (region 5, arrows) to the right of the shoulder area. The VK stain (panel c, showing calcium black) shows two large calcium deposition areas (regions 6 and 7, circled). The area in the plaque shoulder (region 6) shows a particularly dense deposition of calcium (arrows). Because calcium is brittle, dense calcium deposits such as this easily break away during tissue sectioning. We therefore suspect that the missing tissue in this area is dense calcium. The calcium deposition area to the left of the shoulder (region 7) also shows calcium deposition, although it is less dense than in region 6. Moreover, the left side of region 7 stained pink (arrows). A B-Mode image of the excised artery is shown in panel d; enhanced echogenicity and arterial wall shadowing are associated with the plaque (arrows), but other plaque structures are not apparent.

Figure 2 shows a parametric image of ARFI peak displacement (PD). The plaque in the PD image (distal wall, -2 to 10 mm laterally) is spatially matched to the immunohistochemistry shown in Fig. 1. Color in the PD image represents µm, according to the adjacent color bar. First, notice that in the position of foam cell deposition (region 2, arrows), PD are large (~13 µm, dark red) relative to PD achieved in other areas of the plaque. Next, in the position of collagen deposition in the plaque shoulder (region 4, arrows), PDs are relatively small (~4 µm, light blue). Small PDs (~6-7 µm, blue-green) are also apparent in the region corresponding to collagen deposition to the right of the plaque shoulder (region 5, arrows). Finally, in regions 6 and 7, where calcium deposition is extensive, PDs are the relative smallest (~1-3 µm, dark blue), but with focal regions of heightened PD (red, arrows) in region 7 corresponding to the non-calcified tissue. The fibrous cap (region 1) is not differentiated, indicating that it is thin and potentially soft. These data suggest that calcium deposits result in ARFI PDs that are smaller than those associated with other plaque structures including collagen deposits and can therefore be distinguished.

A parametric image of ARFI recovery time (RT) is displayed in Figure 3. The RT and PD images are exactly
registered, so again, the plaque in the RT image is spatially matched to the immunohistochemistry of Fig. 1. Color in the RT image represents ms. At first analysis, it appears that RT is shortest in regions 1 and 3 where elastin is deposited (arrows). However, relative RT comparisons such as these are not possible because the ARFI displacement profiles are corrupted by false peaks. Figure 4 shows three example ARFI displacement profiles from points: 1) in the area of foam cell deposition (region 2), 2) in the area of dense calcium deposition (region 6), and 3) toward the center of the plaque above the calcium deposit (region 5). Note that all three show false peaks (arrows). We hypothesize that the false peaks arise from shear wave reflections off the large calcium deposit boundaries. For the profile derived from the area of foam cells (region 2), shear wave interference occurs after recovery, so the RT measurement is not corrupted (panel a). However, in the dense calcium area, shear wave interference occurs before recovery and corrupts the RT measurement (panel b). A similar trend is observed in region 5 (panel c). It is important to note that such shear wave propagation could be constructive to ARFI imaging of calcium deposits. For example, for an estimated shear wave velocity in a given plaque position, the time delay to the false peak could be used to estimate distance to a calcium deposit. Similarly, for a known calcium deposit position (based on enhanced echogenicity in a match B-Mode image, for instance) an average shear wave velocity could be estimated for the material in a given location in the plaque.

Figure 5 displays a parametric ARFI PD image that was generated by focusing on the far arterial wall (at ~25 mm). In the far wall, a large region of heightened displacement (arrows, dark red, >10 µm) is obvious from -1 to +10 lateral mm, with a narrow band of lower displacement (circled, light blue, ~4 µm) evident from +4 to +5 lateral mm (which spans 4 parallel receive lines). The histology shown in Fig. 5 does not indicate a stiff plaque

MT and SR stains for the near (panels a and b, respectively) and far (panels c and d, respectively) arterial walls of an excised left iliac artery from a 7 yr 4 mo old pig are shown in Figure 5. The MT stains show red blood cells (arrows, ‘1’) that indicate recent hemorrhage and extensive neovascularization that indicates previous hemorrhages. Disrupted smooth muscle cells in the media support peri-vascularitis and perivascular fibrosis (arrows, ‘2’). Collagen deposition regions (arrows, ‘3’) are obvious; however, H&E stains (data not shown) indicate that the collagen is dead tissue and is therefore unlikely to be mechanically coupled. Finally, a small (0.75 cm wide and 0.2 cm tall) calcium deposit is evident in the near wall (panel b, circled, ‘4’). A microcalcification is apparent on the far wall (panel d, circle). The B-Mode image (panel e) shows greater echogenicity in the position of the calcification (circled), but other plaque structures are not readily apparent.
Fig. 6: Parametric ARFI PD image showing shadowing (circled) caused by a small calcium deposit in the near wall (arrow).

Figure 7 displays immunohistochemistry from a 7 yr 2 mo old pig. A raised focal plaque is obvious, with a small calcium deposit (circled) near a thin fibrous cap lacking collagen ('1', arrows) and surrounded by foam cells ('2', arrows). Importantly, this calcium deposit position is believed to degrade the mechanical stability of the plaque (Li et al. 2007). The matched B-Mode image (panel c) shows a spot of increased echo brightness in the position of the calcium deposit (circled), but the fibrous cap and foam cell area are not delineated.

Figure 8 illustrates the corresponding parametric ARFI PD image. A point of relatively small displacement is obvious in the position of the calcium deposit (circled), while the largest displacements are achieved in the position of the foam cells surrounding the calcium deposit (red, ~10 µm, arrows, '2'). Small displacements are also observed in the position of collagen deposition (blue, ~5 µm, arrows, '3'). In this example, the ARFI PD image differentiates the calcium deposit as well as other plaque structures.

IV. Conclusions: We have observed four impacts of calcium deposits on ARFI imaging of atherosclerotic plaques. First, ARFI-induced peak displacements are smaller in the regions of calcium deposits, which supports their differentiation in parametric ARFI recovery time images. Second, presumed shear wave reflections off calcium deposits disrupt estimates of time to recovery from ARFI induced displacement; however, these shear wave reflection may be exploited to indicate calcium position and/or surrounding tissue composition. Third, calcium deposits can result in acoustic shadowing, which would obscure tissue mechanical property below the calcium deposit. Finally, we have also observed different ARFI displacement profile shapes in regions of calcium deposits, but for brevity, these data were not discussed in this paper. In concert with B-Mode ultrasound, which is useful for identifying calcium deposits, we hypothesize that ARFI imaging is relevant to detecting calcium deposits and determining their position relative to other plaque structures.

Acknowledgements: We thank Siemens Medical Solutions USA, Inc., Ultrasound Division for in kind support. This work was supported by NIH grants 2K12HD001441–06, P20RR0207764–01, T32HL069768–05, and AHA grant 0765330U.
Fig. 8: Parametric ARFI PD image showing the smallest displacement in the calcium deposit (circled), surrounded by large displacement in foam cells (2) and adjacent to small displacement in collagen.

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