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iCONCEPTS

CONCEPTS ON THE VERGE OF TRANSLATION

IVUS Detection of Vasa Vasorum Blood Flow Distribution in Coronary Artery Vessel Wall

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There is an increased body of evidence to suggest that the vasa vasorum play a major role in the progression and complications of vulnerable plaque leading to acute coronary syndrome. We propose that detecting changes in the flow in the vascular wall by intravascular ultrasound signals can quantify the presence of vasa vasorum. The results obtained in a porcine model of atherosclerosis suggest that intravascular ultrasound-based estimates of blood flow in the arterial wall can be used in vivo in a clinical research setting to establish the density of vasa vasorum as an indicator of plaque vulnerability. (J Am Coll Cardiol Img 2012;5:935–40) © 2012 by the American College of Cardiology Foundation

The role of vasa vasorum in the pathogenesis and complications of coronary artery disease continues to emerge. Micro-computed tomography (MCT) and other methods have demonstrated that there is proliferation of vasa vasorum early in the development of an atherosclerotic lesion and that this might contribute to plaque rupture (1). The increased density of vasa vasorum due to the angiogenesis and that these new vessels are more fragile increases the chances of hemorrhage from rupture of these new vessels. Thus, there is growing interest to detect the presence of vasa vasorum in the vascular wall and in the atherosclerotic lesion (mainly in the coronary circulation) at an early stage in vivo. Motivated by this reasoning, we explored the concept of the use of intramural blood flow via intravascular ultrasound (IVUS) imaging to detect vasa vasorum density in the arterial wall. Therefore, total flow (increased velocity × area of flow) within the arterial wall should be equivalent to the density of vasa vasorum in the imaged arterial wall. To evaluate this approach we performed both in vivo IVUS with intramural flow capability in coronary arteries of pigs and ex vivo assessment of the vasa vasorum with MCT. Moreover, we enhanced heterogeneity of intramural blood flow distribution by em-

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bolizing some of the vasa vasorum, to provide a range of vasa vasorum densities over which we could compare the IVUS intramural flow and MCT estimates.

After approval from the Mayo Foundation Institutional Animal Care and Use Committee, we employed a porcine model of coronary atherosclerosis to evaluate the feasibility of the *iConcept* proposal. In 7 domestic female cross-bred swine a selective coronary artery catheterization and microembolization procedure was performed as described previously (2). All pigs were anesthetized, heparinized, intubated, and ventilated. A guide catheter was placed into the left anterior descending coronary artery (LAD), and then a 3-F catheter was introduced and advanced until its tip was positioned in the LAD. Then a suspension of 5,000 gold-coated, 100-μm-diameter microspheres (BioPal, Worchester, Massachusetts) was infused into the left coronary artery. After the microembolization procedure, the pigs were allowed to recover and received a high-cholesterol diet, which consisted of 15% lard and 2% cholesterol (Harlan Laboratories, Madison, Wisconsin) for an additional 3 months. Next, the pigs were again anesthetized, and an IVUS imaging catheter (Eagle Eye Gold catheter, Volcano Corporation, Rancho Cordova, California) was introduced and advanced until its tip was positioned in the distal LAD. The catheter tip contained a miniature, multi-element, solid state array ultrasound transducer operating at a frequency of 20 MHz. Then the catheter was connected to an automated pull-back device (Trak Back II, Volcano Corporation) and pulled back at a constant speed of 1 mm/s. A patient interface module connected to the ultrasound array excited the transducer elements to transmit ultrasonic energy to the surrounding tissue; it also amplified and processed the resultant echo signals from the transducer and sent these to the system console (In-Vision System, Volcano Corporation). To visualize blood flow in the coronary artery wall due to vasa vasorum, the specially developed IVUS system (ChromaFlo, Volcano Corporation) was used. This program compared temporally and spatially sequential images along the axis of the artery. Any differences in the position of echogenic regions between images of the tissue surrounding the coronary artery are assumed to be due to blood flow in the arterial wall. The software then colorized the de-correlation rate (i.e., blood flow speed) as a red overlay on the IVUS anatomic image displayed in axial and longitudinal views (3). The resulting "AVI-movie" files were transformed into a stack of transaxial "tif" images (MATLAB, Natick, Massachusetts). These were displayed with an image analysis program (Analyze 9.0, Biomedical Imaging Resource, Mayo Clinic, Rochester, Minnesota) as illustrated in Figure 1. The individual cross-sectional images along the arteries were analyzed individually by creating a region-of-interest (ROI) that encompassed the vessel wall. To ensure that the entire arterial wall was included in the ROI, the radius of the lumen (r) was measured. This measurement then defined the diameter of the annular ROI surrounding the arterial lumen. By creating a binary image of the pixels with flow signal it was possible to sample just the red pixels and from them calculate the total flow value by summing the intensity of the red signal at each pixel. This summation within the ROI represented the total blood flow within the vessel wall (i.e., within the vasa vasorum). The total red signal of the red pixels (per mm^2) was then plotted as a function of axial distance along the coronary artery, thereby creating a vasa vasorum density profile along the axial length of the coronary artery. The microembolization of vasa vasorum produced local regions of reduced blood flow in the coronary artery vessel wall. After the IVUS procedure a midline sternotomy was performed to allow access to the LAD. Then radiopaque contrast dye (Novaplus Omnipaque, GE Healthcare, Princeton, New Jersey) was injected into the proximal LAD, and immediately after the injection an approximately 5-cm-long segment of the LAD was harvested. This involved cutting free the segment with a margin well outside the adventitia to protect and preserve all structures of the vessel wall. This isolated specimen was then snap-frozen. Once frozen the specimens were stored for subsequent scanning with cryostatic MCT. Subsequently, the frozen 5-cm-long specimen was cut into several 2-cm-long segments. This cutting process caused some damage at the ends of each segment. Those individual specimens were scanned as described previously (4). The stack of transaxial tomographic images (side dimension of the cubic voxels was 18-μm, 16-bit gray scale) was displayed and analyzed with image analysis software (Analyze 9.0, Biomedical Imaging Resource, Mayo Clinic). The CT gray scale values were expressed in units of 1,000/cm.

Within the CT images of the arteries, segments of at least 10-mm length, with clearly distinguishable arterial wall, were identified for further analy-

ABBREVIATONS AND ACRONYMS

CMR = cardiac magnetic resonance

CT = computed tomography

IVUS = intravascular ultrasound

LAD = left anterior descending coronary artery

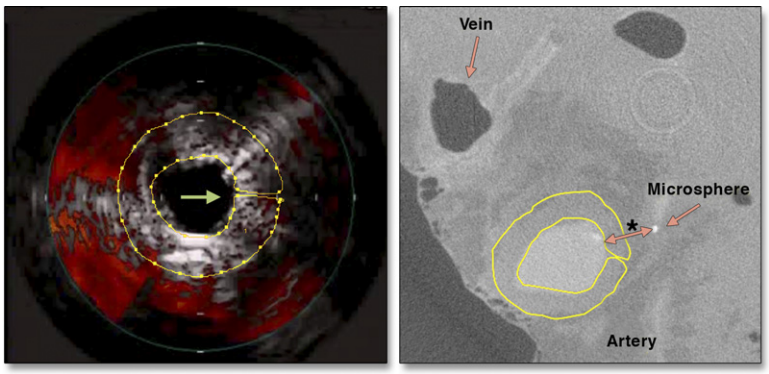

Figure 1. IVUS and MCT Images of Coronary Arteries

Left panel is a single cross-sectional intravascular ultrasound (IVUS) image of a left anterior descending coronary artery. The **white radial lines** represent the tissue of the arterial wall, and the **red features** represent the IVUS-based vessel wall flow assessment (ChromaFlo, Volcano Corporation) data. The **yellow concentric circles** represent the arterial lumen and abluminal adventitial surfaces. The **area between the circles** is the region of interest that was sampled for quantitating the ChromaFlo signal in the arterial wall and was used as the index of vasa vasorum density within the wall. The **right panel** is a cryostatic micro-computed tomography (CT) image of approximately the same arterial cross-section shown in the **left panel**. The **white area** is the intravascular contrast agent within the left anterior descending coronary artery lumen. The **yellow region** of interest outlines the arterial wall. The increase in radiopacity above background radiopacity in this region of interest was used as an index of the blood volume within the vasa vasorum.

sis. The images of the 18-μm-thick cross-sectional slices within the segment (on average 950 slices/specimen) were analyzed individually by creating a ROI that encompassed the entire vessel wall, similar to the analysis of the IVUS flow datasets. Within this ROI the average CT-number was calculated, and this value was plotted as a function of distance along the luminal axis of the arterial segment. This generated an "opacification profile" along the luminal axis of the segment that conveyed regions of varied perfusion within the arterial wall as illustrated in Figure 1. The location of the MCT image data was co-registered with the IVUS flow image data by virtue of the branch points visualized in both images as illustrated in Figure 2. The average values for each of the CT slices in the specimen were averaged and compared with the average value of the IVUS slices in those slices corresponding to the arterial segment scanned with the MCT. Hence, the number of data points is equal to the number of LAD arterial segments scanned. The data are presented as mean ± SD for all arteries. The statistical method used was the regression coefficient (R^2) computed with Microsoft Excel 2003 (Microsoft, Redmond, Washington).

As illustrated in Figure 2, the variation of intramural blood flow and MCT contrast in the arterial vessel wall matched qualitatively quite well. Figure 3 shows a linear relationship (R^2 ranges between 0.90 and 0.96) between CT-number values obtained by cryo-MCT and the vasa vasorum density obtained by IVUS image analysis in each of the 6 animals. However, because there are different amounts of contrast within the arterial wall due to different coronary flow rates and slightly different delays between injection of contrast and harvesting, the CT gray-scale value to intramural flow intensity ratios varies between specimens. Figure 4 shows a comparison of a MCT image of a coronary artery injected with Microfil (thereby showing individual vasa vasorum) and an IVUS pull-back performed on that same artery in vivo before harvesting that artery for MCT scanning.

The *iConcept* study demonstrates the ability to detect and potentially quantify the degree of the vasa vasorum in the coronary vascular wall in vivo. The current concept might emerge as a potential tool to detect early plaque development and vulnerability in vivo in human. The current study uses existing intravascular technology and expends its application for the detection of vulnerable plaque. A previous study (5) using a similar IVUS-based flow measurement to estimate the density of vasa vasorum in arterial walls has several differences that likely explain the contradictory outcome of that study. It made the assumption that the method can provide the actual visualization of the individual vasa vasorum lumen cross-sections. The current study extends this observation and demonstrates that the measurements should not focus on actual imaging of the vasa vasorum, because the vessels of

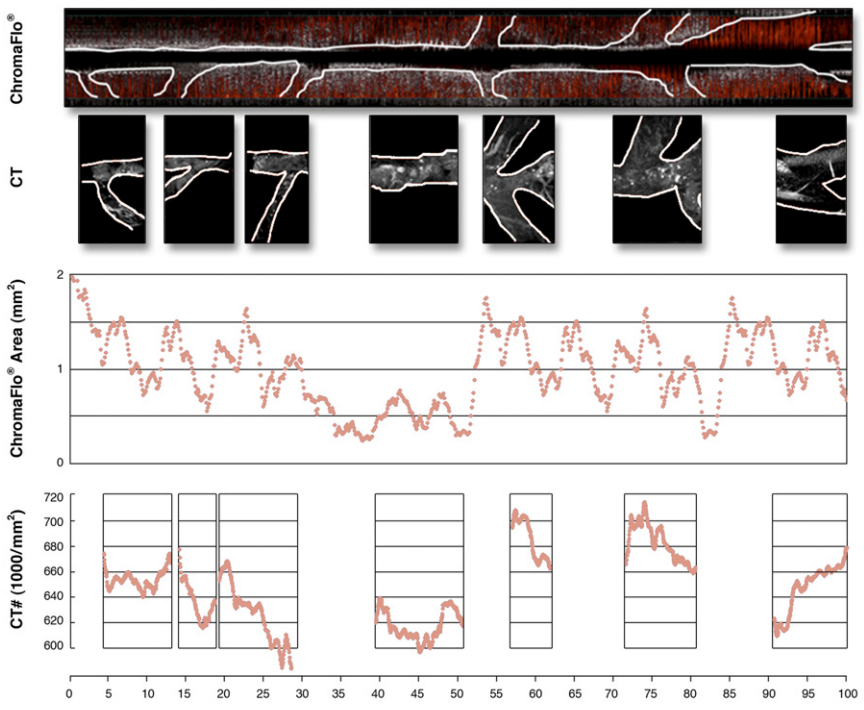

Figure 2. Comparison of Coronary Wall IVUS and MCT Perfusion

Upper-most panel is a longitudinal section along one left anterior descending coronary artery coronary artery computed from the stack of intravascular ultrasound (IVUS) cross-sections obtained during the pull back of the IVUS catheter. The left anterior descending coronary artery lumen and the lumen of the major branches are outlined. The sequence of panels immediately below are the longitudinal sections computed from the 3-dimensional cryo-micro-computed tomography (MCT) image data. The lumens and its major branches are also outlined. The **bright white spots** in the arterial wall are the embolized microspheres. The MCT images are interrupted at the locations where the frozen artery was cut and some local damage occurred. The **lower panels** are the IVUS-based vessel wall flow assessment and MCT opacity increase at each cross-sectional location along the length of the artery.

interest are <100 μm in lumen diameter. Thus, we used the sum of the blood flow within vasa vasorum to quantify the total vasa vasorum flow rather than attempt to spatially resolve the vasa vasorum, and we used MCT imaging—a powerful method for in vitro detection and quantification of the 3D network of vasa vasorum—to compare our IVUS measurements. Interventional selective coronary angiography and CT as well as cardiac magnetic resonance (CMR) angiography methods are not capable of detecting very early lesions that do not have narrowing of the lumen. Currently, multi-slice CT, CMR, IVUS, or optical coherence tomography are used to evaluate coronary artery wall pathology. However, the IVUS and optical coherence tomography methods, although providing important information about changes in the material content in the arterial wall, have not been successful in quantifying the density of vasa vasorum in the arterial wall. However, before any noninvasive approach that quantitates density of vasa vasorum can be implemented, it must be validated. An invasive method that can quantitate the density of vasa vasorum in the coronary artery wall would be acceptable for this purpose. This method would thereby provide an objective method for evaluating noninvasive imaging methods developed to detect early atherosclerotic changes in humans. Because the spatial distribution of vasa vasorum in these pigs was heterogeneous, the good correlation between the IVUS and MCT-based data could be fortuitous, although unlikely to be so in all 6 specimens examined.

The rationale for using the density of vasa vasorum as an indicator of early atherosclerosis is 2-fold. First, it seems to be a direct indicator of the reaction of the arterial wall to early accumulation of fatty materials. Second, the increased volume of blood in the vasa vasorum as well as the increased leakiness of the new vasa vasorum, provide a basis

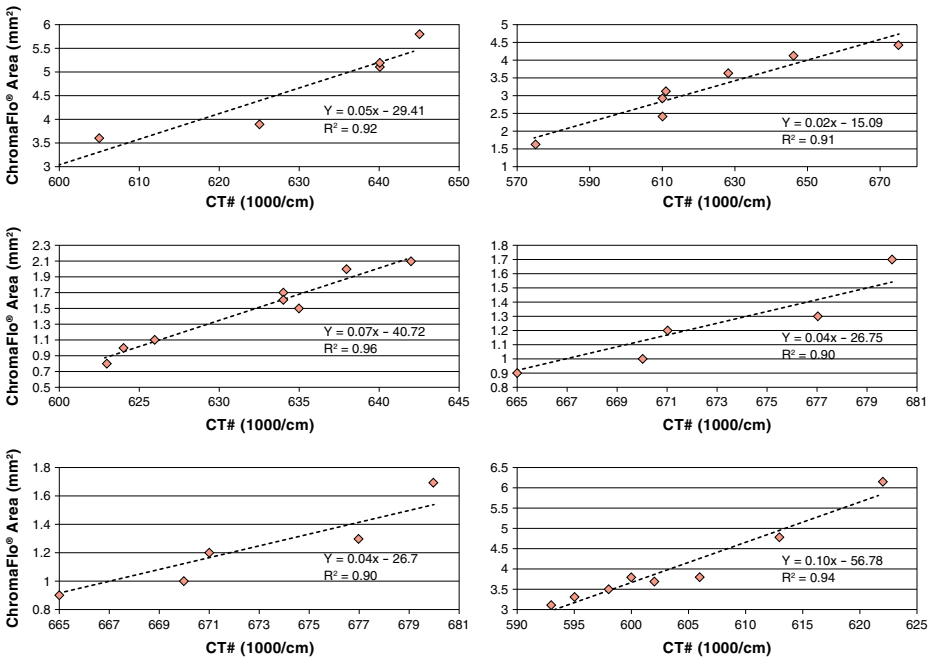

Figure 3. Comparison of IVUS "Flow" Versus MCT Contrast Opacity Values

Plots for each of the 6 pigs. Computed tomography number (CT#) obtained from the cryo-MCT contrast density data plotted versus the IVUS-based vessel wall flow assessment area obtained by the IVUS technique. Abbreviations as in Figure 2.

for specific signals in CT and CMR images. The demonstration of the vasa vasorum in the vascular wall might potentially have implications for future therapeutic approach. In summary, the current study demonstrated a high and significant correlation between the in vitro and the in vivo methods, such that this IVUS-based approach is an excellent candidate for assessing early athero-

Figure 4. Comparison of MCT Anatomy and IVUS Perfusion

Left upper panel is a volume rendered display of an MCT image of a contrast (Microfil)-injected epicardial coronary artery and its surrounding vasa vasorum (VV). **Left lower panel** is a plot of the amount of contrast around the epicardial lumen at up to 1 lumen diameter distance for each CT slice along the axis of the artery. **Right upper panel** is an IVUS pull-back image with **(red)** IVUS-based vessel wall flow signal in the arterial wall region. **Right lower panel** is a plot of the IVUS-based vessel wall flow assessment signal in each cross-section along the length of the artery. Abbreviations as in Figure 2.

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sclerosis changes during clinically indicated selective coronary catheterization and as a means of calibrating noninvasive methods for detection of early atherosclerosis.

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