atherosclerosis

Atherosclerosis newsletter

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Volume 309, Issue September 2020

Volume 310, Issue October 2020

Issues 309 and 310 of *Atherosclerosis* contain several articles on imaging. Several of them describe the results of studies investigating the performance of cardiac computed tomography or optical coherence tomography in the prediction of cardiovascular events. In addition, novel technological developments are reported that improve the visualization of plaques, analyse the metabolic activity of macrophages in atherosclerotic plaques, and record the uptake of macromolecules into the arterial wall.

Predictors of coronary artery calcium incidence and progression: The Brazilian Longitudinal Study of Adult Health (ELSA-Brasil)

Coronary artery calcium (CAC) assessed by non-contrast cardiac computed tomography (CT) is a well-established marker of the total burden of coronary atherosclerosis, independently predictive of atherosclerotic cardiovascular disease (ASCVD) events and cardiovascular mortality. Although it is known that CAC values vary according to race and ethnic background, previous studies of CAC progression have focused predominantly on North American and European populations. Cardoso et al. investigated the risk factors for CAC incidence and progression in the Brazilian population (characterized by a high level of genetic admixture), specifically, a subcohort of the Longitudinal Study of Adult Health (ELSA-Brasil).

Individuals with no prior cardiovascular disease (self-identified as white, brown, black, Asian and Indigenous) and two CAC measurements were included in the analysis. Incident CAC was defined as baseline CAC=0 followed by CAC >0 at the second assessment. CAC progression was defined according to multiple published criteria. Logistic and linear regression analyses were performed to identify risk factors for CAC incidence and progression.

The mean period between CAC assessments was 5.1 ± 0.9 years. CAC incidence occurred in 282/2127 individuals with baseline CAC=0. CAC progression occurred in 319/580 participants with baseline CAC >0. Risk factors for CAC incidence included older age, male sex, white race, hypertension, diabetes, higher body mass index (BMI), smoking, lower high density lipoprotein cholesterol (HDL-C),

higher low density lipoprotein cholesterol (LDL-C) and triglycerides, and metabolic syndrome. Older age and elevated LDL-C were associated with CAC incidence, but not progression. Risk factors consistently associated with CAC progression were hypertension, diabetes, hypertriglyceridemia, and metabolic syndrome. On interaction testing, these four risk factors were more strongly associated with CAC progression as compared to CAC incidence.

CAC incidence was associated with multiple traditional risk factors, whereas the only risk factors associated with CAC progression ere hypertension, diabetes, hypertriglyceridemia, and metabolic syndrome.

Prognostic significance of subtle coronary calcification in patients with zero coronary artery calcium score: From the CONFIRM registry

Coronary artery calcium (CAC) assessed on computed tomography (CT) is a marker of atherosclerosis. The Agatston CAC score (CACS), the most commonly used measurement of the global burden of CAC, is based on both the total area and the maximal density of coronary calcification. Many studies have consistently demonstrated that CACS allows for excellent risk stratification of patients with coronary artery disease (CAD). However, it may fail to identify small or less dense coronary calcification that can be detected on coronary CT angiography (CCTA). Han et al. investigated the prevalence and prognostic importance of subtle calcified plaques on CCTA among individuals with CACS 0.

Patients from The Coronary CT Angiography Evaluation for Clinical Outcomes: An International Multicenter (CONFIRM) registry, without known CAD who underwent CAC scan and CCTA, were evaluated. CACS was categorized as 0, 1–10, 11–100, 101–400, and >400. Patients with CACS 0 were stratified according to the visual presence of coronary plaques on CCTA. Plaque composition was categorized as non-calcified (NCP), mixed (MP) and calcified (CP). The primary outcome was a major adverse cardiac event (MACE) defined as death and myocardial infarction.

Of 4049 patients, 1741 had CACS 0. NCP and plaques that contained calcium (MP or CP) were detected by CCTA in 110 patients and 64 patients, respectively. During a 5.6-year median follow-up, 413 MACE events occurred. Patients with CACS 0 and MP/CP detected by CCTA had similar MACE risk compared to patients with CACS 1–10. In patients with CACS 0, after adjustment for risk factors and symptom, MP/CP was associated with an increased MACE risk compared to those with normal CCTA.

A small but non-negligible proportion of patients with CACS 0 had identifiable coronary calcification, associated with increased MACE risk. Modifying CAC image acquisition and/or scoring methods could improve the detection of subtle coronary calcification.

Association between coronary atherosclerotic burden and all-cause mortality among patients undergoing exercise *versus* pharmacologic stress-rest SPECT myocardial perfusion imaging

When patients with suspected coronary artery disease (CAD) are referred for stress single-photon-emission-computer-tomography (SPECT) myocardial perfusion imaging (MPI) and cannot exercise, pharmacologic stress testing is performed using vasodilator agents or dobutamine. Consistent evidence has established that patients who require pharmacologic testing in lieu of exercise are at substantially increased risk for the subsequent development of cardiac and non-cardiac events. However, the mechanisms underlying this increased risk are not well delineated. To test whether increased atherosclerotic burden accounts for this increased risk, Rozanski et al. assessed the association between coronary artery calcium (CAC) scores and mortality risk among patients undergoing exercise *versus* pharmacologic SPECT MPI.

All-cause mortality in 2151 patients, followed for 12.2 ± 3.4 years after stress-rest SPECT-MPI and CAC scanning within 3 months of each other, was assessed. Patients were divided according to their mode of stress testing (exercise or pharmacologic). Propensity analysis was performed to create a subgroup of exercise and pharmacologic subgroups with comparable age, symptoms, and coronary risk factors.

Despite greater age and worse clinical profiles, pharmacologic and exercise patients had similar CAC scores. However, the hazard ratio for mortality was substantially greater among pharmacologic patients. For each level of CAC abnormality, pharmacologic patients had >2-fold increased risk adjusted hazard ratio for all-mortality risk. Among propensity-matched exercise *versus* pharmacologic patients, the same findings were observed.

In patients referred for stress-rest SPECT-MPI and CAC scoring, pharmacologic patients have substantially increased mortality risk compared to exercise patients, despite having comparable levels of coronary atherosclerosis.

Coronary artery calcium progresses rapidly and discriminates incident cardiovascular events in chronic kidney disease regardless of diabetes: The Multi-Ethnic Study of Atherosclerosis (MESA)

Chronic kidney disease (CKD) is associated with a high prevalence of cardiovascular disease (CVD) events. Clinicians are skeptical about the prognostic role of coronary artery calcium (CAC) in chronic kidney disease (CKD) due to confounding by the altered milieu of calcium/phosphorus metabolism. Shroff et al. assessed the prognostic utility of coronary artery calcium (CAC) scores in discriminating incident CVD events among subpopulations of CKD, particularly those without diabetes mellitus (DM).

Using the Multi-Ethnic Study of Atherosclerosis (MESA), 4 groups based on present/absent CKD/diabetes were defined (CKD-/DM-, CKD-/DM+, CKD+/DM-, CKD+/DM+). Baseline and follow-up CAC measurements were performed, and association between CAC and incident CVD events in a median follow-up of 13 years was evaluated using proportional hazards regression analysis adjusting for demographics, clinical, and biomarker variables.

Prevalence of CKD and DM in the cohort was 13% and 12.5%, respectively. Annual progression in adjusted median CAC score was 24.8%, 27.9%, 26.7%, 36.8% and unadjusted cumulative incident CVD rates were 12.6%, 22.3%, 23.1%, 39.8% for CKD-/DM-, CKD-/DM+, CKD+/DM-, CKD+/DM+, respectively. After full adjustment, hazard ratios (HRs) for incident CVD events were 1.25 CKD-/DM+, 1.10 CKD+/DM- and 2.18 CKD+/DM+. Using CKD-/DM-/baseline CAC = 0 referent, adjusted HRs for incident CVD in CKD+/DM- were 1.30, 2.05, and 4.15 for baseline CAC = 0, 1–100, and >300 Agatston units, respectively, while for CKD+/DM+, adjusted HRs were 3.15, 3.56, 7.90, respectively.

These results suggest that CAC provides incremental prognostic information to predict incident CVD events in CKD regardless of DM. Moreover, baseline CAC categories discriminate incident CVD among CKD without DM, which may have implications in individualizing approach to primary prevention in this high-risk population. These findings are particularly relevant in CKD without diabetes mellitus, in whom lipid lowering therapies are typically underutilized.

Prognostic impact of healed coronary plaque in non-culprit lesions assessed by optical coherence tomography

Previous studies have demonstrated that the main contributors to plaque progression are recurrent silent plaque rupture and subsequent healing or silent plaque erosion and thrombosis. An *ex vivo* study demonstrated that healed plaque (HP) detected by optical coherence tomography (OCT) showed high positive and negative predictive values versus histology. In addition, an *in vivo* OCT study revealed that HP in non-culprit plaques in acute coronary syndrome (ACS) patients was associated with vulnerable plaque morphology; however, clinical data focusing on HP in untreated (non-culprit) segments including stable patients and their impact on subsequent clinical outcomes are scarce. Usui et al. investigated the characteristics and prognostic impact of HP detected by OCT in non-culprit segments in treated vessels.

OCT analysis included HP having a different optical intensity with clear demarcation from underlying plaque, thin-cap fibroatheroma (TCFA), and minimal lumen area. Non-culprit lesion (NCL) was defined as a plaque with >90° arc of disease, length ≥2 mm, and location >5 mm from the stent edges. Major adverse cardiac event (MACE) included cardiac death, myocardial infarction (MI), or ischemia-driven revascularization (IDR).

A total of 726 NCLs in 538 patients who underwent percutaneous coronary intervention with evaluable non-culprit segments by OCT were studied. Prevalence of HP was 17.8% per lesion and 21.9% per patient. At a median follow-up of 2.2 years, there were 65 NCL-related MACE events, including 6 MIs and 65 IDRs of which 87.7% had a stable presentation. The presence of untreated HP was positively correlated with subsequent NCL-related MACE. There were 16 IDRs with stable angina occurring at a specific OCT-imaged NCL where an untreated HP was positively associated with subsequent NCL-related MACE along with TCFA and minimal lumen area <3.5 mm^2.

An OCT-detected HP in an NCL is a marker for future (mostly) stable non-culprit-related MACE at both a patient- and lesion-level.

These results are discussed in more details in the editorial by Majeed et al.

Two-photon excited fluorescence (TPEF) may be useful to identify macrophage subsets based on their metabolic activity and cellular responses in atherosclerotic plaques

Atherosclerosis is characterized by the formation of lipid plaques within the arterial wall. In such plaques, the massive and continuous recruitment of circulating monocyte-derived macrophages induces inflammation, leading to plaque destabilization and rupture. Plaque vulnerability is linked to the presence of (i) a large lipid core that contains necrotic, “foamy” macrophages (FMs), (ii) a thin fibrous cap that cannot limit the prothrombotic lipid core, and potentially (iii) an imbalance between inflammatory and immunoregulatory macrophages. These opposite macrophage functions rely on the use of different energy pathways (glycolysis and oxidative phosphorylation, respectively) that may lead to different levels of the auto-fluorescent cofactors nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD). Borowczyk et al. hypothesized that high-resolution two-photon excited autofluorescence (TPEF) imaging of these cofactors may be used to monitor the metabolic activity and cellular responses of macrophages in atherosclerotic plaques.

Different models of human FMs were generated by exposure to acetylated or oxidized low-density lipoproteins (LDL), with/without human carotid extract (CE). Their phenotype and optical properties were compared with those of extremely polarized macrophages, inflammatory M1 (MLPS+IFNγ) and immunoregulatory M2 (MIL4+IL13).

These FM models displayed an intermediate phenotype with low levels of M1 and M2 “specific” markers. Moreover, the NADH and FAD autofluorescence profiles of FMoxLDL ± CE cells were significantly distinct from those of M1 and M2 macrophages.

TPEF imaging may be useful to follow the metabolic activity and cellular responses of the different macrophage subtypes present in atherosclerotic plaques in order to detect vulnerable areas.

Photon-counting CT with tungsten as contrast medium: Experimental evidence of vessel lumen and plaque visualization

Computed tomography angiography (CTA) using iodinated contrast media is the most widely applied modality for imaging carotid artery disease as both the vessel lumen and atherosclerotic plaques can be visualized and characterized with high accuracy. CTA of the neck and brain vessels is thus routinely performed in patients with suspicion of cerebrovascular stroke.
However, image quality of CTA may be negatively impacted by calcified plaques that lead to blooming and beam-hardening artefacts. Sartoretti et al. investigated the potential of a preclinical photon-counting detector CT (PCT) scanner with an experimental tungsten-based contrast medium for carotid artery imaging.

A carotid artery specimen was imaged on a PCT system using the multi-energy bin option at two radiation doses with iodine and tungsten as contrast media at equal mass-concentrations. Standard CT, virtual non-calcium (VNCa) and calcium-only images were reconstructed. Subjective image quality was rated using histology as reference. Noise and attenuation measurements were performed. Simulations were conducted to assess the material-decomposition efficiency for different object diameters.

Image quality on VNCa images was significantly higher for tungsten at lower dose. Noise was significantly lower at both dose levels for tungsten VNCa images as compared to iodine images. Simulations indicated improved material-decomposition efficiency for tungsten than for iodine pronounced at smaller object diameters.

PCT employing the multi-energy bin option in combination with tungsten as contrast media enables improved carotid artery imaging with respect to lumen and plaque visualization and image noise.

3D confocal microscope imaging of macromolecule uptake in the intact brachiocephalic artery

Elevated uptake of plasma macromolecules by the arterial wall is an early event in atherogenesis. Existing optical techniques for detecting macromolecular tracers in the wall have poor depth penetration and hence require *en face* imaging of flattened arterial segments. Imaging uptake in undistorted curved and branched vessels would be useful in understanding disease development. In the present work, Dazzi et al. used benzyl alcohol:benzyl benzoate (BABB) to overcome the inherently poor penetration of light into the arterial tissue.

Depth penetration was increased by applying optical clearing techniques. The rat aorto-brachiocephalic junction was imaged intact by confocal microscopy after being exposed to circulating rhodamine-labelled albumin *in vivo*, fixed *in situ*, excised and then cleared with BABB. Tracer uptake was mapped on a 3D surface mesh of the arterial geometry.

Tracer fluorescence was detectable throughout the wall closest to the objective lens and, despite a vessel diameter of 1 mm, in the wall on the other side of the artery, across the lumen. By tile scanning, tracer concentrations were mapped in the aorta, the brachiocephalic artery and their junction without opening or flattening either vessel. Optical clearing was also shown to be compatible with immunofluorescent staining and imaging of experimental atherosclerosis.

The technique obviates the need for labour-intensive sample preparation associated with standard *en face* imaging. More importantly, it preserves arterial geometry, facilitating co-localisation of uptake maps with maps of biomechanical factors, which typically exist on 3D surface meshes. It will allow correlation of haemodynamic wall shear stress with macromolecule permeability more accurately in regions of high curvature or branching, such as the coronary arteries.