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Vascular Calcification: The evolving relationship of vascular calcification to major acute coronary events

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RUNNING TITLE: Vascular Calcification

Learning Objectives

On successful completion of this activity participants should be able to (1) describe the pathogenesis of atherosclerosis; (2) understand the importance of the necrotic core in the genesis of microcalcification in the atheroma; and (3) distinguish between the prognostic importance of dense calcification and multiple small foci of calcification adjacent to lipid lakes.

ABSTRACT

Calcification in a coronary artery is accepted as definite evidence of coronary atherosclerosis. The extent and density of calcification, as combined in the Agatston score, is associated with the risk of a patient experiencing a major acute coronary event (MACE). Atherosclerosis occurs because damaged endothelial cells allow low density lipoprotein cholesterol (LDLc) to leak into subintimal tissue. Proteoglycans in subendothelial collagen have a high affinity for LDLc, retaining the lipoprotein cholesterol complex. As the endothelial damage is repaired, the subintimal LDLc is trapped. Retained LDLc induces an inflammatory response in the overlying endothelium, causing the endothelium to express chemotactic peptides. Chemotactic peptides attract circulating monocytes, which follow the concentration gradient, enter the tissue and become tissue macrophages to phagocytize and digest the irritating LDLc in the atheroma. In the process of digesting LDLc, enzymes in the macrophages oxidize the LDLc complex. Oxidized LDL is toxic to macrophages; when present in sufficient quantity, it may cause death of macrophages, contributing to inflammation in the atheroma. In a necrotic inflammatory lesion, the regulatory mechanisms that control tissue concentrations of calcium and phosphorous are lost, allowing the solubility product of calcium phosphate to be exceeded, resulting in the formation of microscopic calcium-phosphate crystals. With ongoing inflammation, additional calcium-phosphate crystals are formed, which may aggregate. When these aggregated calcium phosphate crystals exceed ~ 0.2mm, the lesions become visible on clinical CT as coronary calcifications. Serial gated CT scans of the heart have demonstrated that once formed, CT visible calcifications do not decrease significantly in size, but may increase.

Although dystrophic vascular calcification is a 'tombstone,' it does not identify the lesions likely to cause MACE. Atheroma that are actively undergoing calcification are the most likely to cause major acute events, and molecular PET/CT imaging with ionic ^{18}F fluoride identifies such lesions. Recent data suggest that ^{18}F-fluoride imaging may be a sensitive and specific marker of lesions likely to cause MACE. A multicenter trial is needed to define whether this marker identifies patients at high risk of MACE.

NATURAL HISTORY OF ATHEROMA AND VASCULAR CALCIFICATION

Vascular calcification can occur in the intima and in the media of arteries. Medial calcification is more common in peripheral vessels and is often associated with elevated levels of calcium or phosphorous, as seen in chronic kidney disease and hyperparathyroidism. Although lesions in the media are palpable at autopsy, they rarely encroach on the vessel lumen (1). On the other hand, intimal calcification is associated with atherosclerosis. Atherosclerosis is a multifocal disease of uncertain etiology characterized by the accumulation of low density lipoprotein cholesterol (LDLc) beneath the intima of vessels, causing an inflammatory lesion, called an atheroma. In patients with persistently elevated circulating levels of LDLc (1), atherosclerosis progresses over decades with enlargement of existing lesions and formation of new lesions. Atheroma cause single or multiple narrowings of the vascular lumen. A combination of luminal narrowing (with associated turbulence of blood flowing past the irregular surface), may cause local increases in pressure on the vessel wall. If there is a severely inflamed atheroma with a thin fibrous cap separating the necrotic material in the atheroma from the flowing blood, the fibrous cap may rupture (2), releasing thrombogenic material into the vascular lumen, resulting in the formation of a thrombus. If the thrombus is large enough to impede blood flow, it may result in a major acute cardiovascular event (MACE), such as myocardial ischemia, myocardial infarction, transient ischemic attacks and stroke. Although the prevalence of atheroma is very high, the incidence of clinical events is extremely low: “On the basis of autopsy findings, it is likely that in the game of atheroma roulette fewer than 1% of atheroma ruptures result in a clinical event.”(3)

In spite of the low rate of clinical events caused by ruptured atheromas, atherosclerosis is so prevalent that cardiovascular disease accounted for about one third of all deaths in the US in 2016, with coronary heart disease accounting for 360,000 deaths (4). In spite of ‘major anti-smoking campaigns, medications to control high blood pressure and cholesterol’ (5) coronary heart disease remains a major cause of death in middle aged Americans. To reduce the

mortality from coronary disease, multiple approaches have been developed to identify patients at risk for MACE.

Beginning with the Framingham heart study in the 1950's, several clinical and laboratory factors were identified to predict the likelihood that a patient will have a clinical cardiac event. For example, to define the perioperative risk of MACE in patients requiring urgent surgery, the revised cardiac risk index described by Lee et al (6) is often used as a first step (TABLE 1). For patients with less urgent needs, there are multiple approaches, including online risk calculators (7) and guidelines to predict the risk that a patient will experience MACE in the next 10 years. Patients at high risk are considered for additional clinical and imaging evaluation, such as a high sensitivity C-reactive protein blood test and/or a gated, non-contrast CT scan (8) to identify coronary artery calcification as an indication of the burden of coronary artery disease (FIGURE 1 A and B).

Calcification in an atheroma starts in the inflamed necrotic core. The necrotic core contains a 'witches brew' of oxidized lipoprotein cholesterol, lipid laden macrophages (foam cells), as well as dead and dying macrophages and smooth muscle cells. Histopathology of atheroma demonstrates numerous microcalcifications 2-15 micrometers [1 micrometer=0.001 mm] in diameter in areas of necrosis (Figure 2 A-D). When hyperlipidemia persists, especially in the presence of other cardiovascular risk factors (e.g., smoking, diabetes and uncontrolled hypertension), the necrotic core enlarges, and both the number and size of the calcifications increase. Prior to the advent of digital radiography, coronary calcification was detected by cardiologists during x-ray fluoroscopy, where foci of coronary calcification would 'dance' on the screen in synchrony with the patient's heart beat. Today, coronary artery calcification is identified and quantified on non-contrast, EKG gated computed tomography. A focus of calcification must be larger than 0.1mm to be detected on a clinical multidetector CT scan. The focus must also exceed a density of 130 Hounsfield units and have a volume of at least 3 pixels to meet criteria for a significant calcification. Although several approaches to calculate a coronary calcification score have been developed, (9) the scoring system used most often, the Agatston score, combines the density and extent of calcification in each coronary artery into a

single score and sums the value to provide a score of the 'burden of coronary atherosclerosis' for that patient (10).

EVOLUTION OF ATHEROMA

In addition to forming a semi-selective barrier for retaining macromolecules in the vasculature, vascular endothelium (11) participates in "…immune reactions, vascular repair, and metabolism of bioactive molecules." Under physiologic conditions, endothelial cells serve as a barrier, preventing the low-density lipoprotein cholesterol complex (LDLc) in blood from reaching subintimal tissue. Endothelial cells, however, may be injured by trauma, infection, inflammation, or autoimmune processes. Injured endothelium is permeable to LDLc, allowing the lipoprotein cholesterol complex to come into contact with subendothelial collagen containing proteoglycans, where it is retained. As the endothelial damage is repaired by adjacent endothelial cells, the subendothelial LDLc is trapped. The cholesterol in trapped LDLc may crystalize (12), causing volume expansion and local inflammation (13). The inflamed endothelial cells express chemotactic peptides (14)(15) that attract circulating monocytes to enter the subendothelial tissue. Infiltrating monocytes differentiate into tissue macrophages to phagocytize the lipoprotein-lipid complex. During phagocytosis and digestion of the lipoprotein bound cholesterol, the macrophages produce enzymes which oxidize the LDLc complex. Oxidized LDL is toxic to the macrophage and when present in sufficient quantity, cause the macrophage to die. Other cells in the atheroma, such as smooth muscle cells may also die in the intense inflammatory milieu.

Early atheroma show only a low level of inflammation. In this phase death of macrophages often occurs by organized mechanisms, such as apoptosis (16). When cells die by apoptosis the remnants of the dead cell are phagocytized by adjacent cells, minimizing residual inflammation in the lesion. In larger, severely inflamed, lesions, cells die not only by apoptosis, but also by disorganized mechanisms, such as necroptosis and necrosis. The latter allow remnants of dead cells to remain in the lesion, increasing inflammation. This type of inefficient cleanup of dead cell remnants, called inefficient efferocytosis (17), adds the detritus of the dead and dying cells to the necrotic core of an atheroma, enlarging the lesion, increasing inflammation, and providing a nidus for dystrophic calcification (18)(19) (FIGURE 3).

Treatment of hyperlipidemia with lipid lowering medications, such as statin drugs and PCSK9 inhibitors, are currently the standards of care. In addition to the pharmacologic agents,

there are lifestyle changes that patients can make to reduce inflammation in atheroma, including: exercise, maintaining a normal BMI, controlling blood sugar in diabetic patients, and eating a Mediterranean diet. If the patient continues pursuing a proatherogenic life style, however, inflammation in atheroma will likely persist or increase.

FORMATION OF A CALCIFICATION

In healthy cells the local concentrations of calcium (20) and phosphorous (21), are tightly regulated, minimizing the possibility of forming unwanted calcium-phosphate crystals. The remnants of dead and dying cells in the necrotic core of an inflamed atheroma, on the other hand, do not have the control mechanisms required to regulate the concentration of calcium and phosphate, allowing these ions to exceed the solubility product to form calcium phosphate crystals. For example, enzymes such as alkaline phosphatase, adenosine triphosphatase, and reactive oxygen species available (22) in the necrotic core create free phosphate from the breakdown of larger molecules. Micro-vesicles (23)(24)(25) produced in the process of inflammation, apoptosis, necrotic cell disintegration, and budding of the cell plasma membrane (26) may serve as scaffolding for calcium-phosphate crystal formation(27). Depending on the cells providing the micro-vesicles, the particles may contain substances that facilitate formation of calcium phosphate crystals or agents that inhibit vascular calcification (see below). When calcium phosphate crystals form in the necrotic core, they may also trap other ions, such as magnesium.

CHARACTERIZATION OF CALCIFICATION

A non-contrast gated CT can be quantified to provide a value that reflects a combination of the extent and density of coronary arterial calcifications (10)(28)(29)(30). The ~2-15 micrometer crystals initially formed in the necrotic core (Figure 2) are too small to be visible on clinical CT, which has a spatial resolution ~ 0.1 mm. Over time, in the presence of persistent hyperlipidemia, new microcrystals form in the necrotic core and the microcrystals aggregate. Studies using electron beam CT almost 3 decades ago and recent studies with non-contrast EKG gated CT suggested that patients' with higher Agatston scores had a greater risk of MACE (31). Recent studies, however, suggest (32) that the volume and density of coronary calcification

have different prognostic implications. A ***higher density*** calcification may reflect lower lipid content or prior subclinical plaque rupture with healing, suggesting that heavily calcified lesions are stable (33), and carry a better prognosis. In contrast, multiple small foci of ***lower density*** calcification, particularly in the region of lipid pools (i.e., spotty or fragmented calcification), suggest a less stable lesion with a higher risk for MACE.

Several large trials, such as the PROSPECT ('Providing Regional Observations to Study Predictors of Events in the Coronary Tree') sub study (34) also reported that culprit lesions contained *less* calcium than stable lesions, supporting the observation that dense calcification is associated with lesion stabilization (35).

In the lab, the stability of an atheroma can be evaluated by mechanical stress analysis (36). For instance, proximity of a rigid inclusion (microcalcification) to a compliant inclusion (lipid, usually in the necrotic core) near the surface of an atheroma enlarges the area of increased wall stress compared with the deposition of either inclusion alone (37). Other factors influencing the stress on the cap of an atheroma include:

- where the rigid inclusion is located (near the surface or deep in the lesion);
- size of the rigid inclusion (calcification);
- whether there is a single calcification or multiple calcific foci near each other;
- orientation of the foci with reference to the direction of blood flow.

Most microcalcifications in an atheroma occur near dead or dying macrophages, deep in the necrotic lipid core, rarely with inclusion in the fibrous cap (where the increased stress can lead to plaque rupture).

Using a high-resolution laboratory CT scanner to determine the number of calcifications in human coronary artery specimens, investigators (38) observed ***thousands*** of microcalcifications (97% of them less than 0.2 mm) in 62 coronary artery fibroatheromas, with 81 microcalcifications in the fibrous cap of 9 atheromas. Three-dimensional finite element analysis showed that peak circumferential stress on the fibrous cap of atheromas could increase 5-fold if two microcalcifications in the cap were a critical distance from each other and

oriented along the tensile axis of blood flow; this level of stress would be more than sufficient to rupture the cap. On the other hand, when the microcalcifications were located within the viscous necrotic core, separated from the cap, the calcific foci were not likely to increase shear stress on the cap (39).

STABILITY OF CALCIFICATION

Macroscopic coronary artery calcifications are irreversible. They are the end result of a pathologic process due to dysregulated or inappropriate stimuli (40). Whether the calcification is active or passive is unclear (41).

Serial CT measurements have shown that calcifications are either stable or increase over time.(42)(43) (Although some studies have reported a slight decrease in calcification in some lesions, these small reductions are likely due to technical factors, rather than a real reduction in the amount of calcium at the site. (42) Persistence of calcification is also supported by a long term animal study wherein monkeys first received an atherogenic diet followed by prolonged feeding of a diet aimed at the regression of atherosclerosis (44). Histopathology of the coronary arteries in animals sacrificed during the atherogenic diet phase demonstrated intra- and extracellular calcium particles, usually adjacent to large lipid pools. Animals sacrificed 3.5 years later, after completing the atherosclerosis regression diet, had regions of calcification in the absence of lipid pools, supporting the concept that calcifications do not regress.

MECHANISMS TO LIMIT VASCULAR CALCIFICATION

Under physiologic conditions, multiple factors inhibit or prevent (45)(46) macroscopic vascular calcification; for instance:

a. Fetuin A (a 59-kDa glycoprotein. . This glycoprotein, produced by the liver, binds small calcium phosphate crystals and forms calciprotein particles which are phagocytized by macrophages.
b. Osteocalcin. This vitamin K dependent matrix protein, produced by osteoblasts, inhibits apatite crystal growth.

c. Matrix gamma carboxyglutamate Gla protein (MGP). This 14 kDa protein absorbs crystalline calcium phosphate. Both osteocalcin and MGP require activation by vitamin K (47). MGP is synthesized by vascular smooth muscle cells, chondrocytes, endothelial cells and fibroblasts and is secreted locally. MGP binds calcium phosphate by chelating calcium and phosphate ions, as well as crystals (46). When vitamin K levels are reduced or absent, MGP remains inactive, as do other clotting factors such as prothrombin and factors VII, IX and X (48)(49). The vitamin K antagonist coumadin (warfarin) inhibits vitamin K activation by inhibiting the vitamin K epoxide reductase complex 1 (VKORC1). In the absence of active vitamin K, these important substances to limit vascular calcification are inactive, resulting in a surprising and undesirable increase in vascular calcification as seen in patients treated with coumadin. In a 66 patient randomized trial comparing changes in coronary artery calcification in patients with atrial fibrillation treated with warfarin vs apixaban (50), the calcified and low attenuation plaque volume in the group randomized to warfarin was higher compared to apixaban. Similarly, an analysis of 8 prospective randomized trials using intravascular ultrasound (IVUS) demonstrated a significant increase in the annualized calcium index of patients treated with warfarin compared to those without warfarin (51).

AGING AND VASCULAR CALCIFICATION

Aging contributes to both increased stiffness of conduit arteries (52) and increased atherosclerosis (53). Aging occurs, in part, because adult cells are not immortal. Hayflick (54) demonstrated that normal human diploid cells have a limited capacity to replicate in-vitro (approximately 40 generations). At the end of their reproductive capacity, the final generation of cells are not replaced. This limitation is possibly due in part to the reduction in the length of telomeres with each division. With age there is also a gradual increase in collagen and a decrease in elastin in the major conduit vessels. Part of the elastin decrease is due to a 'fatigue failure' (55), due to the repeated expansion and contraction of conduit vessels with each heartbeat. Age is associated with an increase in the risk for coronary artery disease in the absence of risk factors such as a history of smoking, hypertension, hyperlipidemia, or glucose

intolerance (56). Advanced age is also associated with "vessel dilatation, wall thickening, reorganization of cellular and extracellular matrix and an increase in subintimal space due to the accumulation of collagen and mononuclear cells."(56) The extracellular matrix is rich in glycosaminoglycans, which retain LDL/cholesterol that penetrates the more permeable, senescent (57)(58) endothelial cells (59).

Role of Statins

Therapy with statins decreases circulating levels of LDLc, but may paradoxically also increase coronary vascular calcification (60), probably by stabilizing atheromas. Lower levels of circulating LDLc effectively decrease subintimal lipid accumulation, resulting in reduced inflammation, decreased size of the necrotic core and possibly increased calcification of the necrotic core[Error! Bookmark not defined.].

POTENTIAL ROLE OF MOLECULAR IMAGING

Identifying patients at risk of sudden death remains a major challenge (5). A recent editorial (61) summarized information from multiple studies relating MACE to imaging findings using both invasive intravascular ultrasound and optical coherence tomography as well as contrast enhanced CT imaging. Positive predictive values of these imaging studies ranged from 4% to 22%, which is not sufficient to promulgate a percutaneous intervention (such as stenting) in an asymptomatic patient (even if there is a low risk of adverse events). A test with a much higher positive predictive value would be much more compelling to advocate treating a patient with no symptoms (62).

Several molecular imaging techniques have been proposed to localize and characterize atheroma (62), including: radiolabeled autologous low density lipoprotein (63), radiolabeled antibodies recognizing oxidized LDL (64), radiolabeled somatostatin receptor antagonists (to image somatostatin receptors expressed by inflamed atheroma) (65), radiotracers localizing in hypoxic atheroma (66), 2-fluoro-2-deoxyglucose (FDG) (67) to identify focal vascular inflammation, and recently, imaging with F-18 fluoride (68) to identify active calcification. Although FDG localizes in the carotid arteries of patients with symptomatic carotid disease, it

remains unclear whether FDG coronary arterial uptake correlates with coronary events (69). In a direct comparison of FDG to ^{18}F sodium fluoride in patients with myocardial infarction and stable angina, "NaF showed substantially higher uptake in the culprit plaque (70)." Fluoride imaging demonstrated high sensitivity for detecting high-risk lesions that did not concentrate FDG but demonstrated high risk plaque characteristics by optical coherence tomography (OCT), CT and IVUS (71). Vascular fluoride uptake also correlated with the presence of microvessels seen on OCT, suggesting that fluoride avid lesions had increased inflammation. It is conceivable that fluoride imaging may offer the positive predictive value required to consider therapy in patients at high risk for MACE in the absence of clinical symptoms. This question would have to be addressed in a prospective multicenter study.

CONCLUSIONS

Coronary artery calcification seen on non-contrast CT indicates the presence of atherosclerosis. Lesions demonstrating spotty calcification in regions with adjacent pools of lipid are more likely to be unstable. On the other hand, if the calcifications are dense and plate-like, especially in the absence of adjacent lipid pools, the CT findings suggest stable lesions**.** PET/CT imaging using NaF can detect active calcium deposition at multiple vascular beds on a single scan, suggesting that whole body scans can identify high risk lesions in multiple beds.

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TABLE 1

REVISED CARDIAC RISK INDEX (6)

A. High-risk type of surgery (major non-cardiac surgery
B. History of ischemic heart disease
C. History of congestive heart failure
D. History of cerebrovascular disease
E. Preoperative treatment with insulin
F. Preoperative serum creatinine >2.0 mg/dL.

Rates of major perioperative cardiac complication in patients with 0, 1, 2, or > or = 3 of these factors were 0.5%, 1.3%, 4%, and 9%,

FIGURE 1 A

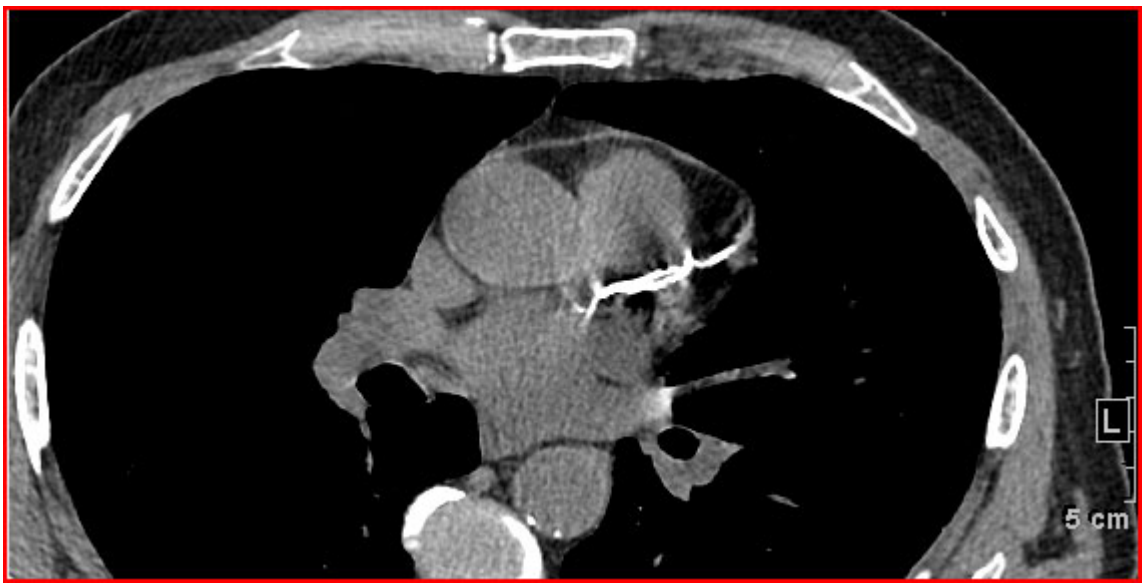


FIGURE 1 B

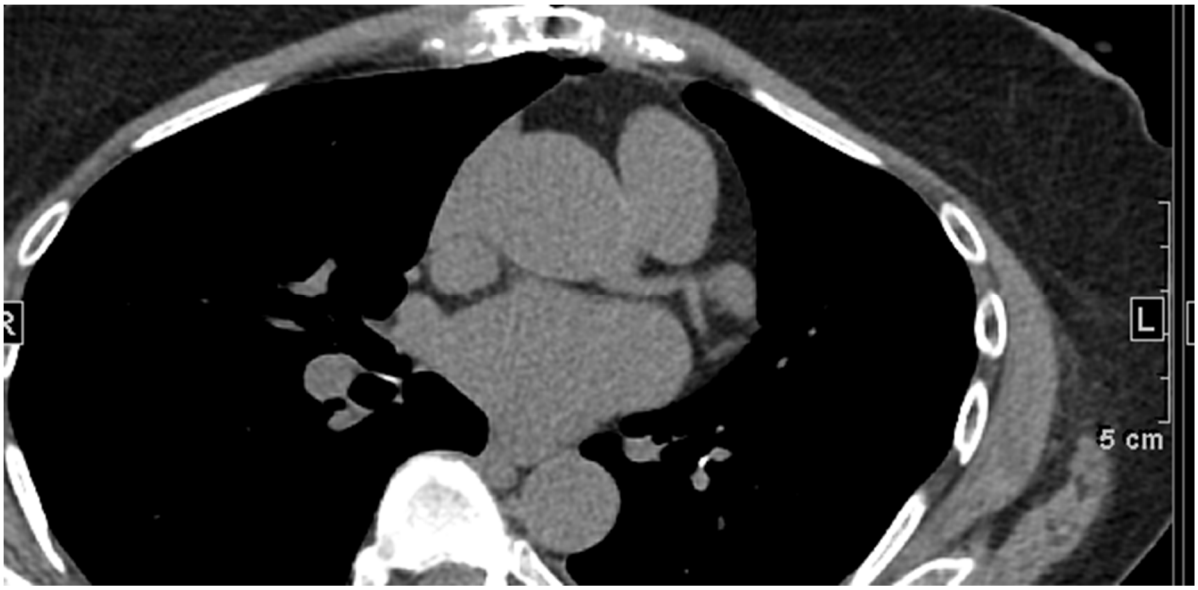
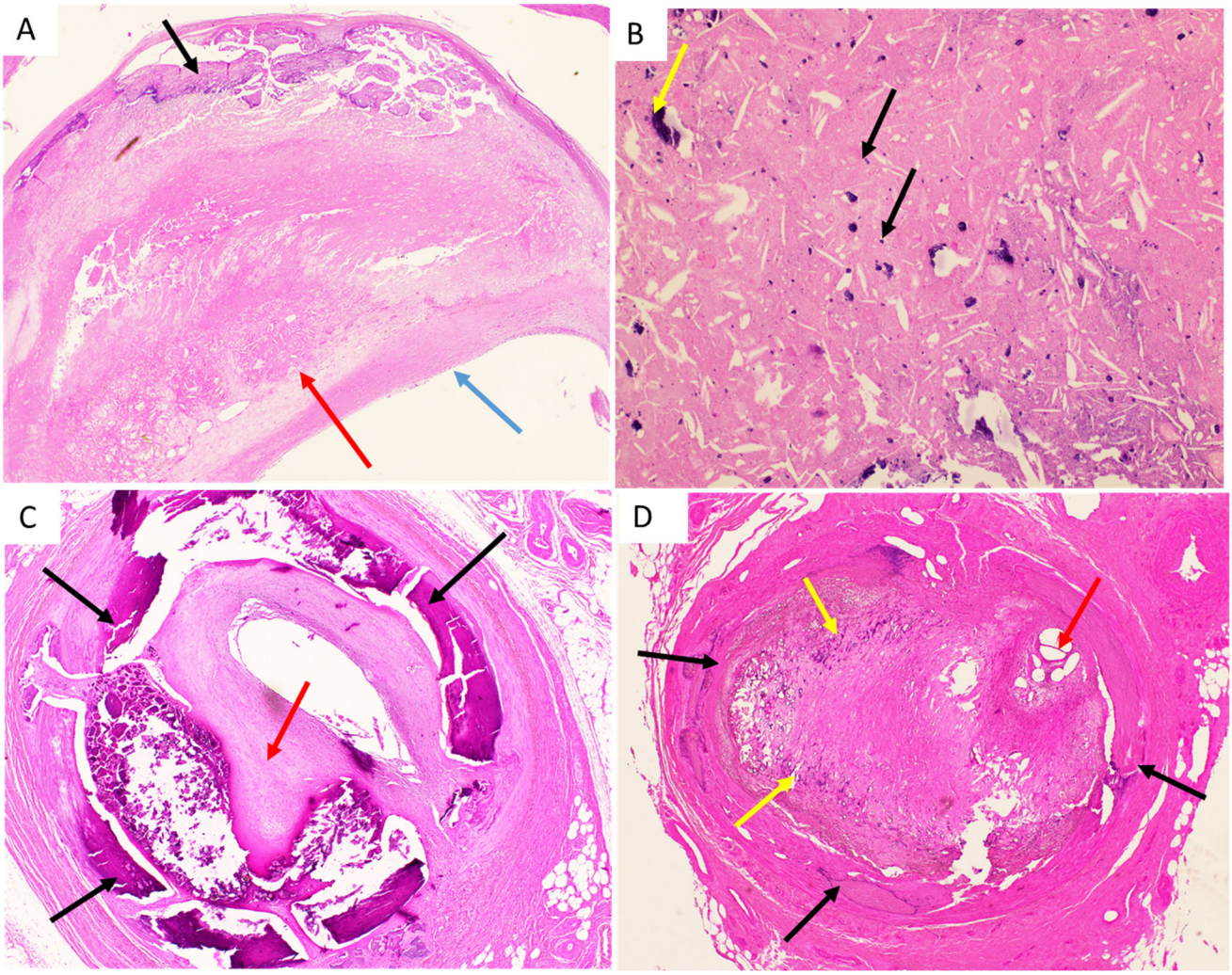


FIGURE 1 A and B, selected gated cardiac CT images of two patients.

A. Coronary calcium score = 3218. The patient is a-70 year old male, ex smoker, with a family history of CAD. The ammonia perfusion scan demonstrated a medium sized area of moderate ischemia in the mid to distal inferolateral wall. In view of extremely high calcium, the amount of ischemia may be underestimated. The ejection fraction was 76%. The stress ECG had significant ST depression in lead 2 and aVf.

B. Coronary calcium score = 0. The patient is a 69 female with a family history of myasthenia gravis. She presented with, increasing dyspnea and chest pain on exertion. Past history of renal cell carcinoma and hypertension.

FIGURE 2



A. Atherosclerotic plaque from a carotid endarterectomy specimen has a fibroatheromatous plaque with a large necrotic core (red arrow), intact fibrous cap (blue arrow) and plate like calcification (black arrow) at the base (away from the luminal aspect) of the plaque. Magnification 2X.

B. A higher magnification of the necrotic core of an atheromatous plaque from another carotid endarterectomy specimen shows punctate calcification (yellow arrow) and microcalcification (black arrows). Magnification 20X.

C. Dorsalis pedis artery from a patient who underwent amputation for critical limb ischemia shows pathological intimal thickening (red arrow) and medial calcification (black arrows) involving almost the entire circumference of the artery. Magnification 20X.

D. Peroneal artery from another patient who underwent amputation for critical limb ischemia shows an atherosclerotic plaque with punctate calcification (yellow arrows), luminal recanalized thrombus (red arrow) and medial calcification (black arrows). Magnification 20X.

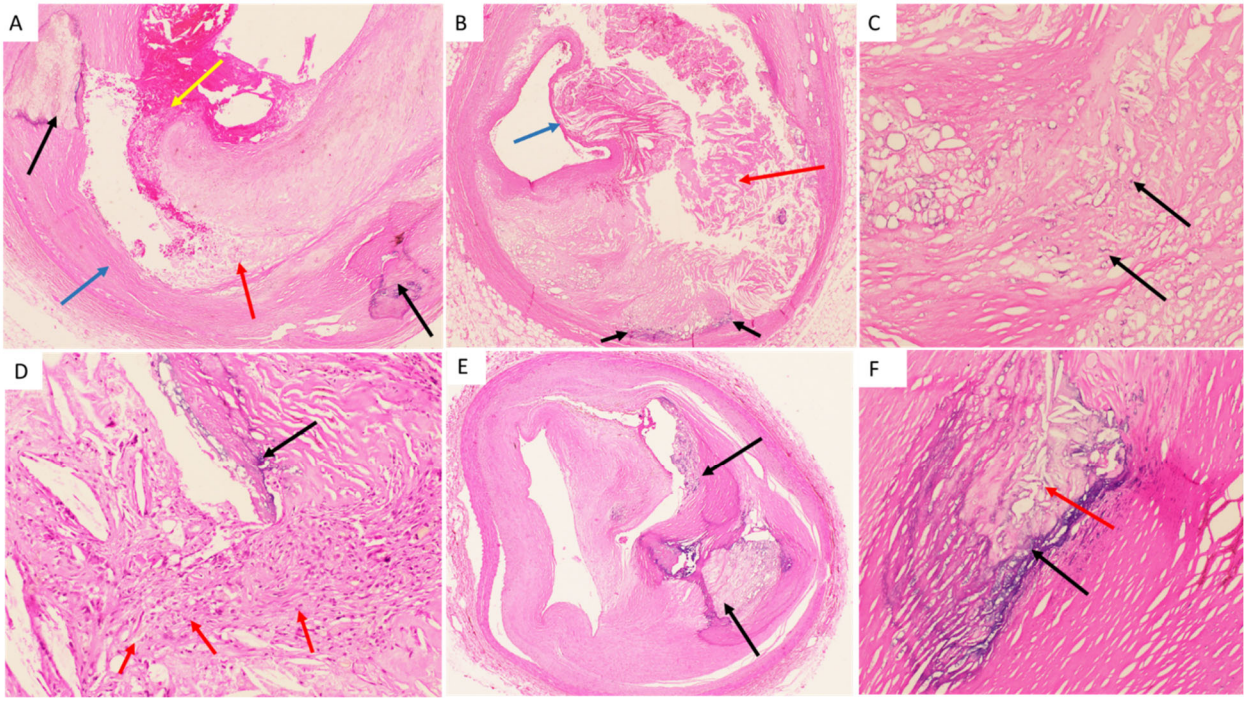


FIGURE 3

A: Coronary artery showing a Fibroatheromatous plaque with rupture of the fibrous cap (yellow arrow) with an acute luminal thrombus that communicates with the necrotic core (red arrow) at the site of rupture. Deep to the necrotic core the plaque is fibrotic (blue arrow) with plate like calcification (black arrow). Magnification 10x.

B. Fibroatheromatous plaque in a coronary artery with a large necrotic core (red arrow), thin fibrous cap (blue arrow) and punctate calcification at the base of the plaque (black arrows). Magnification 4X

C and D are higher magnifications of necrotic core in different atheromatous plaques of coronaries that show calcification in the necrotic core (black arrows). The plaque shown in image C is not associated with inflammation, whereas the plaque shown in image D is associated with significant inflammation (red arrows). Magnification 20X

E: Coronary artery with a fibrocalcific plaque. Arrows point to plate like calcium. Magnification 4X

F. Higher magnification of the plaque shown in image E shows a small residual necrotic core (red arrow) in the center of a predominantly fibrocalcific plaque. Magnification 20X.

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