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REVIEW

STATE-OF-THE-ART PAPER

Coronary Artery Calcification and its Progression

What Does it Really Mean?

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ABSTRACT

Coronary artery calcification is concomitant with the development of advanced atherosclerosis. Coronary artery calcification pathologically begins as microcalcifications (0.5 to 15.0 μm) and grows into larger calcium fragments, which eventually result in sheet-like deposits (>3 mm). This evolution is observed to occur concurrently with the progression of plaque. These fragments and sheets of calcification can be easily identified by radiography as well as by computed tomography and intravascular imaging. Many imaging modalities have proposed spotty calcification to be a predictor of unstable plaque and have suggested more extensive calcification to be associated with stable plaques and perhaps the use of statin therapy. We will review the pathology of coronary calcification in humans with a focus on risk factors, relationship with plaque progression, correlation with plaque (in)stability, and effect of pharmacologic interventions. (J Am Coll Cardiol Img 2018;11:127–42) © 2018 by the American College of Cardiology Foundation.

The presence of calcification has been recognized in atherosclerotic coronary arteries for more than a century. Coronary artery calcification (CAC) implies the presence of coronary artery disease (CAD) irrespective of risk factors or symptoms, is concomitant with the development of advanced atherosclerosis (1), and is an established predictor of future cardiac events (2,3). Generally, CAC correlates with the extent of CAD. The presence and extent of CAC can be assessed by various imaging modalities with computed tomography (CT) having the most available correlation with prognostic outcomes data. Both the extent of calcification as well as its pattern has prognostic implications. In general, spotty calcification is more commonly associated with unstable plaques and extensive calcification more so with stable plaques, but the relationship of CAC to plaque instability is extremely complex and incompletely understood. In this review, we will focus on human CAC from a pathologic standpoint and explore its implications with regards to plaque progression and the relationship of the extent and patterns of calcification to plaque morphology as seen pathologically and radiographically. We will also explore the effects of various principle risk factors and pharmacologic interventions on CAC. Pathophysiological

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ABBREVIATIONS AND ACRONYMS

ACS = acute coronary syndrome(s)

AMI = acute myocardial infarction

BMP = bone morphogenetic protein

CAC = coronary artery calcification

CHD = coronary heart disease

CT = computed tomography

HU = Hounsfield unit

IVUS = intravascular ultrasonography

OCT = optical coherence tomography

OFDI = optical frequency domain imaging

TCFA = thin-cap fibroatheroma

mechanisms of calcification, as understood from correlating pathology and imaging, will be examined to offer a pathological perspective for predicting risk of future events.

TYPES AND PATHOLOGY OF CALCIFICATION IN ATHEROSCLEROSIS

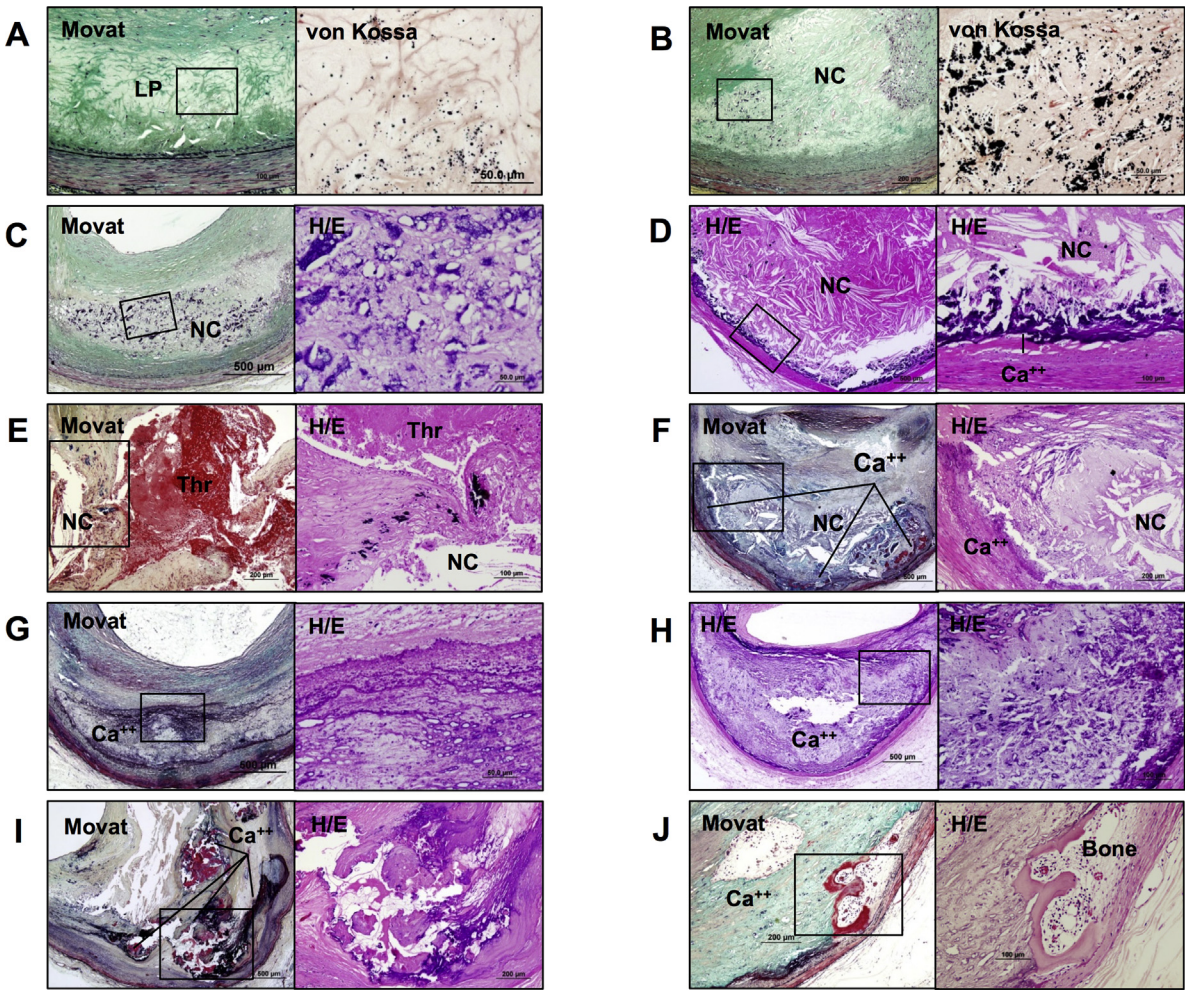
Vascular calcification can be classified into 2 distinct forms depending on its location within the intima (intimal calcification) or in the vascular medial layer. Medial calcification mostly affects the peripheral arteries of the lower extremities, resulting in the loss of elasticity, and is routinely observed in patients with peripheral vascular disease. Furthermore, the progression of arterial medial calcification is reported to be associated with renal failure, hypercalcemia, hyperphosphatemia, parathyroid hormone abnormalities, and duration of dialysis. Meanwhile, intimal calcification is the dominant type of calcification seen in coronary arteries. Therefore, we will mainly focus our discussion on intimal calcification in this article.

The earliest form of CAC is microcalcification seen in lesions with pathological intimal thickening with a size ranging from 0.5 to 15.0 μm (4–6), observed best by special stains for calcium such as von Kossa and Alizarine red (**Figure 1A**). Within the lipid pool, early microcalcification is visible by light microscopy and is thought to originate from smooth muscle cell (SMC) apoptosis (4,6,7). Initial calcification occurring in matrix vesicles with a diameter from 100 to 700 nm can only be observed by electron microscopic examination (8). In addition to SMC apoptosis, macrophage-derived matrix vesicles also play a role in the process of microcalcification (9). SMC apoptosis leads to fine microcalcifications, whereas apoptotic macrophages produce relatively larger punctate appearance (**Figures 1B and 1C**). These calcific deposits are commonly seen in the deeper areas of necrotic core close to internal elastic lamina (**Figure 1D and 1E**). Microcalcifications coalesce into larger masses over time to form speckles and fragments of calcifications. The progression of calcification occurs from the outer rim of the necrotic core into the surrounding collagenous matrix (**Figure 1F**); the central core may or may not become calcified at this stage. Further progression of calcification leads to calcified plaques typically with calcified sheets or plates (>1 quadrant), involving SMCs and collagenous matrix regardless of the necrotic core (**Figure 1G and 1H**). Calcified sheets may fracture leading to the formation of nodular calcification (**Figure 1I**). These nodules may extend into the lumen or the media and can be associated with fibrin deposition. These protuberant nodules can lead to discontinuity of the endothelial lining and underlying collagen matrix, and acute luminal thrombosis. The calcified nodule is the underlying mechanism of acute coronary events in 2% to 7% of coronary artery thrombosis (10) and 4% to 14% of the carotid artery thrombosis (11) in pathological studies.

Bone formation (12) comprising trabecula with marrow space is rarely seen in CAD within calcific regions (**Figure 1J**). When observed, it is usually seen in heavily calcified segments of the arterial wall suggesting that osteogenesis might be exclusively associated with severe arterial calcification. In amputated lower limbs due to peripheral artery disease, the incidence of bone formation has been reported to be as high as 19% (13). It has been suggested that the process of arterial calcification shares some features with skeletal bone formation, including chondrocyte and osteoblast differentiation, mineralization, and bone matrix deposition and resorption. Bone-related proteins such as bone morphogenetic protein (BMP)-1 and BMP-4, bone sialoprotein, osteocalcin, osteonectin, osteopontin, and osteoprotegerin have been identified in calcified arteries (14,15). In early coronary plaques, osteoprotegerin, osteopontin, and matrix Gla protein have been reported at sites of microcalcification; uncarboxylated matrix Gla protein expression is not seen in adaptive intimal thickening (AIT) but evolves with the development of pathologic intimal thickening (PIT) and fibroatheromas (15). BMP-2, BMP-4, osteopontin, and osteonectin are more prevalent in lesions with bone formation in fibrocalcific plaques. Although bone-related protein expression exists from early stages of plaque formation, the precise mechanisms of calcification or osteogenesis in atherosclerosis have remained uncertain.

There are various stimuli in the initiation and progression of calcification which may differ depending upon the stage of plaque as well as the surrounding milieu. As alluded to above, we believe that the death of SMCs is the driving force for early microcalcification (1). This is followed by infiltration of macrophages into the lipid pool which also undergoes cell death and calcification. Cell death provides phospholipid-rich debris that serves to nucleate apatite, a process that starts within lipid pools and progresses with inflammation and further cell death, leading to the development of a necrotic core.

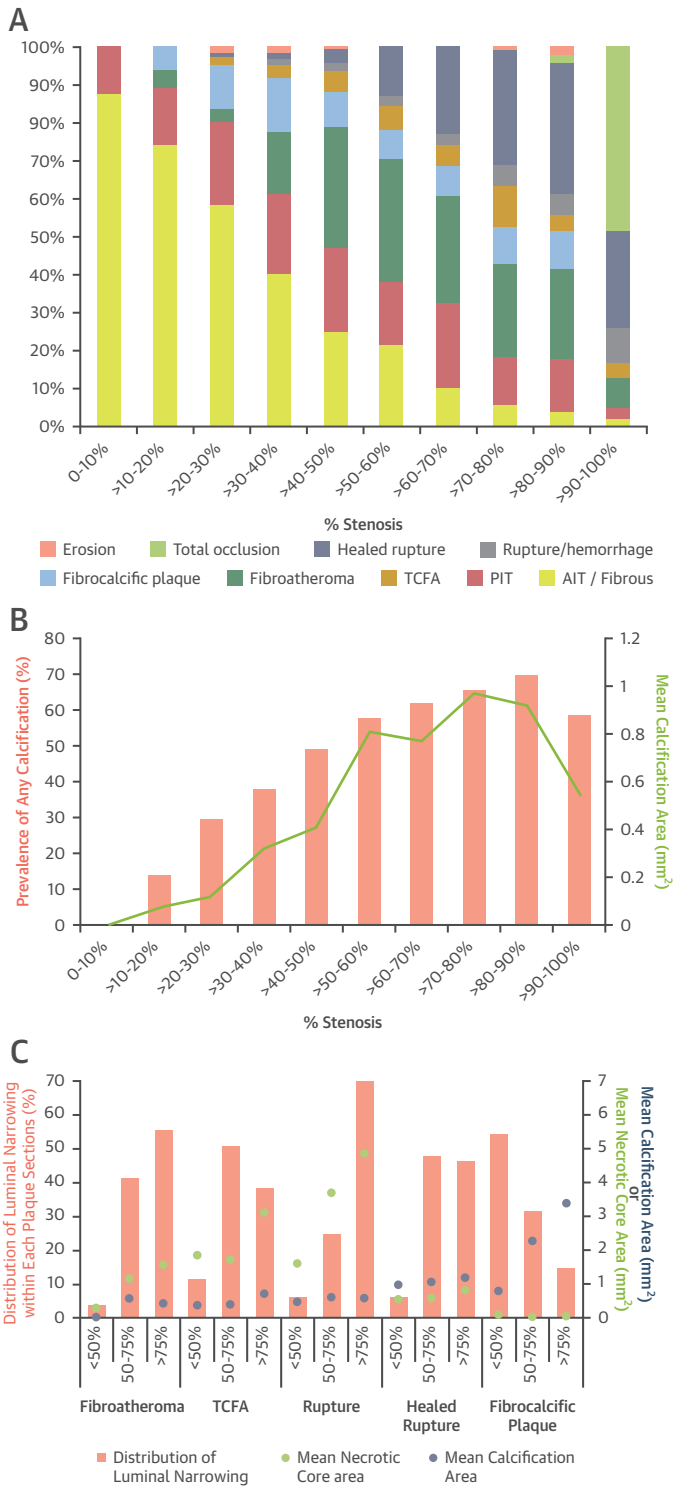
FIGURE 1 **Histologic Progression of Coronary Calcification**



Nondecalcified arterial segments **(A and B)** and decalcified segments **(C to J)** were serially cut for microscopic assessment. **(A)** Pathologic intimal thickening (PIT) is shown characterized by lipid pool (LP) that lacks smooth muscle cells (SMCs). Early microcalcification (≥0.5 µm, typically <15 µm in diameter) likely results from SMC apoptosis and calcification is detected by von Kossa staining within the LP (high-power image of boxed area in the Movat pentachrome-stained image). Early necrotic core (NC) **(B)** not only lacks SMCs but also is infiltrated by macrophages which eventually undergo apoptosis and calcification, which is observed as punctate (≥15 µm) areas of calcification. Von Kossa staining clearly shows relatively larger punctate areas of calcification resulting from macrophage cell death within the NC as compared to microcalcification of dying SMCs. A substantial amount of macrophage calcification can be observed in early NC **(C)**, but the degree of calcification in NC is typically located close to the medial wall where fragmented calcifications can be seen **(D)**. Microcalcification resulting from macrophage and/or SMC death can also be detected within a thin fibrous cap and has been associated with plaque rupture **(E)**. Calcification generally progresses into the surrounding area of the NC **(F)**, which leads to the development of sheets of calcification where both collagen matrix **(G)** and NC itself are calcified **(H)**. Nodular calcification may occur within the plaque in the absence of luminal thrombus and is characterized by breaks in calcified plates with fragments of calcium separated by fibrin **(I)**. Ossification may occur at the edge of an area of sheet calcification **(J)**. Reproduced with permission from Otsuka et al. (1). Ca^{++} = calcium; H/E = hematoxylin-eosin stain; Thr = thrombus.

Environmental factors such as elevated calcium or phosphorus promotes apatite nucleation and crystal growth (16). Although other theories of the mechanisms underlying vascular calcification have been proposed, some are more clearly relevant to the process of medial rather than intimal calcification (17). Loss of inhibitors of mineralization mainly promote medial calcification in animal models, whereas osteogenic mechanisms involving bone forming proteins such as osteopontin, collagen type I, osteoprotegerin, and so on, have been proposed to play a role. However, actual bone formation inside the vessel wall is

FIGURE 2 Relationship of Calcification, Plaque Type, and Percent Stenosis



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rarely observed in human coronary arteries (18,19). How calcium becomes fragmented or sheet calcium is poorly understood, although by the time the latter is observed inflammation is minimal. We believe the development of sheet calcium stabilizes plaques. Although vascular SMCs can undergo osteogenic transformation into osteoblast-like cells, such changes have rarely been observed in human atherosclerosis (16). Factors such as intraplaque hemorrhage have been associated with calcification and may exacerbate its progression, although further work is needed to understand how this might occur (20).

Overall, calcification patterns vary widely depending on the location. The peripheral arteries tend to have greater collagen deposition and calcification and show Mönckeberg type medial calcification in addition to intimal sclerosis. On the other hand, some arterial beds, such as the internal thoracic artery, are reported to be resistant to atherosclerosis and also to calcification (21).

NATURAL PROGRESSION OF CALCIFICATION WITH PLAQUE TYPE

Calcification progresses with plaque type as well as with degree of luminal narrowing (1). Calcification is often absent or minimal in early lesions such as adaptive intimal thickening or pathological intimal thickening. Plaque type and complexity increases (**Figure 2A**) with greater degrees of luminal narrowing as do the prevalence and area of calcification (**Figure 2B**). The pattern and extent of calcification, however, does not correlate linearly with the necrotic core size and tends to differ sharply by plaque type (**Figure 2C**). In fibroatheroma, thin-cap fibroatheroma (TCFA), and rupture, necrotic core area increases progressively but calcification may or may not increase proportionately. However, in healed ruptures and fibrocalcific plaques, calcification area increases out of proportion to necrotic core area as the vessel narrows. In classification by matched radiography and histology, diffuse calcification correlated with sheet calcification by histology and is frequently observed in healed ruptures and fibrocalcific plaques (**Figures 3 and 4**). On the other hand, TCFA and rupture have varying degrees of calcification with less diffuse calcification by radiography (**Figure 3**) and lower sheet calcification by histology (**Figure 4**). Although erosion is 1 of the major causes of coronary thrombosis (22), calcification level is clearly different from TCFA/rupture showing much less advanced calcification.

AGE, SEX, AND CALCIFICATION

It is well known that sex affects the development of atherosclerosis and that in females the disease is delayed by 10 to 15 years compared to males, which is likely due to the protective effects of estrogens in the pre-menopausal years (23). In a sub-study of the Women's Health Initiative, 1,064 females aged 50 to 59 years randomized to estrogen therapy or placebo showed a significantly lower mean CAC score in the estrogen group (83.1) compared to placebo (123.1; $p = 0.02$). The CADRE (Coeur Artères DREpanocytose) study evaluated the extent of CAC in 108 human hearts explanted from victims of sudden cardiac death including 70 men (mean age 50 ± 12 years) and 38 women (mean age 50 ± 12 years) using a semi-quantitative radiographic scale (grade 0 = no calcification; 1 = calcification <40 μm; 2 = calcification >40 μm in diameter involving only 1 quadrant; 3 = calcification >40 μm in 2 quadrants; 4 = calcification >40 μm in 3 quadrants; 5 =calcification >40 μm in all 4 quadrants) (24). When divided by decades, the extent of calcification was greater in males compared to females up to the 6th decade but became similar by the 7th decade (1). We have also observed that the degree of calcification was 3 times greater in postmenopausal females versus premenopausal females (25).

RACE AND CALCIFICATION

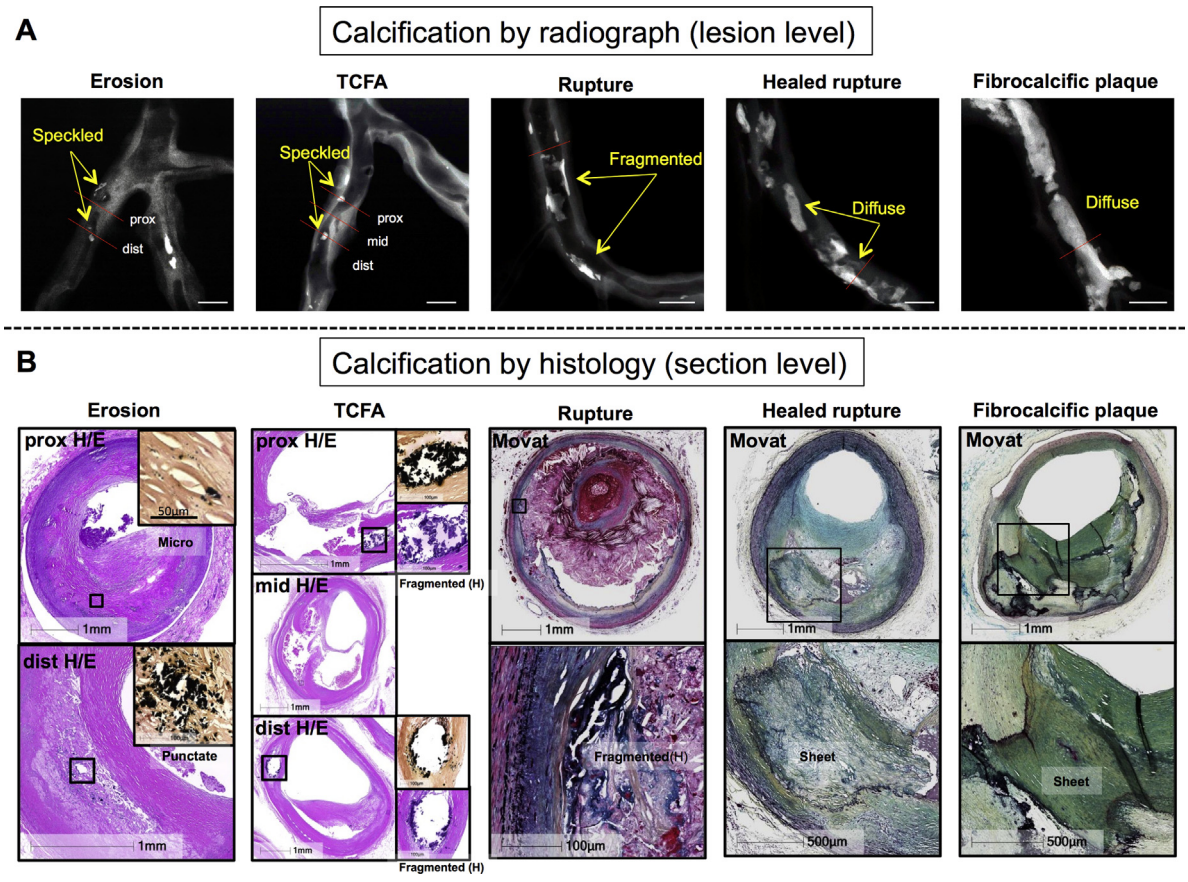
There is a strong racial variation in degree of CAC which might underlie important differences in clinical outcomes. In the MESA (Multi-Ethnic Study of Atherosclerosis) study (26), a total of 6,814 whites, African Americans, Hispanic, and Chinese people aged 45 to 84 years with no history of clinical cardiovascular disease were assessed for coronary calcification. The prevalence of coronary calcification (Agatston score >0) in these 4 ethnic groups in males was 70.4%, 52.1%, 56.5%, and 59.2% ($p < 0.001$), respectively, and in females was 44.6%, 36.5%, 34.9%, and 41.9% ($p < 0.001$), respectively. After adjustment for age, education, lipids, body mass index, smoking, diabetes, hypertension, treatment for hypercholesterolemia, sex, and scanning center, compared with whites, the relative risks for having coronary calcification in women was 0.78 (95% confidence interval [CI]: 0.74 to 0.82) for African Americans, 0.85 (95% CI: 0.79 to 0.91) for Hispanics, and 0.92 (95% CI: 0.85 to 0.99) for Chinese. After similar adjustments, the CAC in those with an Agatston score >0 was greatest among whites, followed by Chinese (77% that of whites; 95% CI: 62% to 96%), Hispanics (74% that of whites; 95% CI: 61% to 90%), and African Americans (69% that of whites; 95% CI: 59% to 80%). Similar to this and other studies (27,28) in CADRE, we have observed that the extent of CAC in explanted hearts was greater in Caucasians versus African Americans for every decade. Several possible explanations have been forwarded for the relatively higher CAC in Caucasians such as the bone mineral density and bone turnover. Bone loss has been associated with abdominal aortic calcification (29). African Americans have higher bone mineral density compared to whites ($p < 0.001$ for total body; $p = 0.0001$ for L2-L4, and $p = 0.0005$ for femur neck region) (27) implying that perhaps lower bone turnover might be the cause of lower arterial calcification. Speculating specific race-related genes to account for these differences, microarray gene expression profiling of peripheral blood leukocytes was undertaken in 119 healthy women (≥50 years old) from the MESA cohort; 48 women had high (>100) and 71 had low (<10) CAC scores. When 17 African Americans were compared with 41 white women with low CAC score, 409 genes were found to be differentially expressed. In addition, 316 genes were differentially expressed between high (>100) and low CAC (<10) score groups. Genes characterized by lower expression in African Americans showed similarly lower expression in individuals with low CAC score (correlation 0.69; $p = 0.002$) (28).

Polymorphisms of the soluble epoxide hydrolase gene were reported to be associated with CAC scores in African-American subjects; although the minor allele frequency was similar between blacks (16.7%) and whites (19.3%). The Arg287Gln polymorphism was a significant independent predictor of both presence and extent of CAC in African-American but not in Caucasian subjects (30). However, the precise genetic impact of race on calcification remains uncertain.

FIGURE 2 Continued

(A) Data are stratified by decades. Used with permission from Burke et al. (1,24). **(B)** Prevalence of various coronary plaque morphologies at 10% incremental cross-sectional area narrowing in sudden coronary death victims. Data presented as each 10% increase in narrowing. Used with permission from Burke et al. (1,24). **(C) Pink bar graph** shows the percent area luminal narrowing divided into severe (>75% cross-sectional area narrowing), moderate (50% to 75%), and mild (<50%) luminal narrowing stratified by plaque type. Mean necrotic core **(yellow circles)** and calcification area **(blue circles)** are also shown for each percent narrowing. The data for fibroatheroma, TCFA, and rupture were obtained (n = 213). Reproduced with permission from Narula et al. (73). The data of healed rupture and fibrocalcific plaque were obtained (n = 30). Reproduced with permission from Burke et al. (24). AIT = adaptive intimal thickening; TCFA = thin-cap fibroatheroma; other abbreviation as in **Figure 1**.

FIGURE 3 Type of Calcification by Radiography and Histology



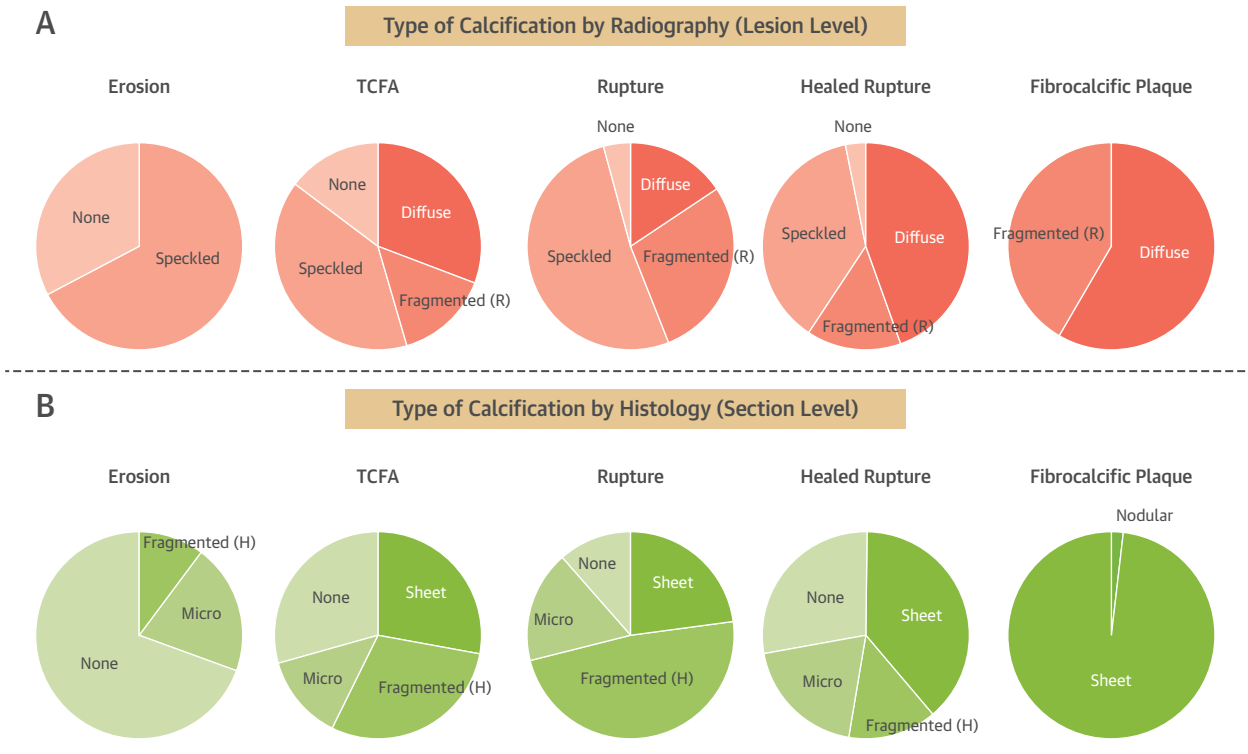
(A) The typical patterns of calcification by radiography for each plaque type. **(B)** Corresponding histology with low- and high-power images of sections taken from the areas shown in radiographs as **red line**. Scale bar **(white line)** in radiograph indicates 5 mm. Microcalcification by histology is invisible by radiograph in proximal section from a case of coronary erosion. Speckled calcification by radiograph corresponds to punctate or fragmented calcification by histology. TCFA lesions shows 3 sites of sectioning and 2 spots (proximal and distal) of speckled calcium with the middle section showing TCFA without calcification. Plaque rupture site shows fragmented calcification by radiograph which corresponds to fragmented calcification by histology. Healed rupture and fibrocalcific plaques sites both showing diffuse calcification by radiograph which correspond to sheet calcification by histology. Dist = distal; Fragmented (H) = fragmented by histology; Mid = middle; Prox = proximal; other abbreviations as in Figures 1 and 2.

DIABETES AND CALCIFICATION

CAC tends to be higher in diabetic patients, which correlates with total plaque burden and represents an independent risk factor for adverse outcomes (31,32). Chronic kidney disease can be comorbid with diabetes is also a risk factor for CAC (33). The role of glycosylated hemoglobin on CAC progression was evaluated in 2,076 patients using noncontrast CT at baseline and after 5 years of follow-up (34), wherein the incident CAC increase was categorized as any (>10 Agatston unit change between examinations) or advanced (>100 Agatston unit change between examinations) (34). Higher glycosylated hemoglobin was associated with any incident CAC progression (RR = 1.51; 95% CI: 1.16 to 1.96), and advanced CAC progression (RR = 2.42; 95% CI: 1.47 to 3.99) (34).

We have previously observed hearts from diabetic subjects and age, race, and sex-matched nondiabetic subjects dying suddenly (35). Total plaque burden and distal plaque burden were significantly greater in type II diabetic subjects versus nondiabetic subjects, which can be partially explained by the fact that the numbers of healed plaque ruptures were greater in type II diabetic subjects (2.6 vs. 1.9; p = 0.04) (36).

FIGURE 4 Types of Calcification on Radiography Versus Histology in Various Plaque Types



(A) The distribution of calcification types by radiograph. **(B)** The distribution of calcification by histology. The data on erosion, TCFA, and healed rupture in **A** were obtained and reproduced with permission from Burke et al. (24). The data of fibrocalcific plaque in **A** were obtained and reproduced with permission from Yahagi et al. (74). The data shown in **B** was obtained and reproduced with permission from Yahagi et al. (16). Fragmented (R) = fragmented by radiograph; other abbreviations as in Figures 2 and 3.

The mean percent calcified area was greater in type II diabetic subjects (12.1%) versus nondiabetic subjects (9.4%; $p = 0.05$). The mean percent plaque area composed of necrotic core was also greater in type II diabetic subjects (11.6%) compared with nondiabetic subjects (9.4%; $p = 0.004$). Macrophage plaque area and T-cell infiltration were significantly greater in diabetic than nondiabetic patients ($p = 0.03$), along with human leukocyte antigen, type DR (HLA-DR) expression ($p < 0.0001$), which indicates the involvement of greater chronic inflammation in diabetic subjects. Expression of receptor for advanced glycation end product was also significantly greater in diabetic subjects ($p = 0.004$) and was associated with apoptotic SMCs and macrophages.

CALCIFICATION WITH PLAQUE STABILITY

CAC is strongly associated with adverse outcomes in all the populations studied so far, and it is a far better marker of future events than all the risk factors or risk equations to date. What is not clear so far, however, is whether this is related to the calcified plaque itself as the source of the events or whether calcified plaques just predict the presence of CAD (hence risk) most accurately. Perhaps the most crucial clinical question regarding CAC is whether its presence predicts plaque instability or stability. Pathologic studies have inherent limitations for answering this question, but a combination of coronary imaging and pathology has provided some preliminary answers. It appears that a physician cannot treat CAC as an all-or-none variable; the type of calcification, its location, its extent, as well as its volume and density have differential effects on clinical risk and outcomes (37). This is what creates the controversy about whether it is the presence of CAC or its progression (implying need for serial measurements clinically) that is most important for predicting risk and outcomes (38,39).

In general, spotty calcification seems to predict plaque instability, whereas heavy calcification correlates with overall plaque burden. Serial intravascular

ultrasound (IVUS) studies have reported that spotty calcification was associated with greater progression of plaque volume compared to noncalcified plaques, whereas heavily calcified plaques were resistant to change in plaque volume (40,41). Spotty calcification by CT angiography was more frequently observed in patients presenting with acute coronary syndrome (ACS) or likely to develop ACS in the short term when the plaques also showed positive remodeling and low-attenuation (42). In our Coronary Artery Sudden Death Registry, we have observed maximum histological calcification in fibrocalcific plaques followed by healed plaque rupture, plaque rupture, TCFA, and minimally in plaque erosion (24) (**Figures 3 and 4**). The relationship between percent stenosis in each of these plaque types, mean calcium area, and necrotic core (**Figure 2C**) revealed that fibrocalcific plaques were more heavily calcified with increasing luminal stenosis followed by healed ruptures. Only mild increases in calcium occurred with luminal narrowing for all other plaque types. Conversely, core area was greater as stenosis increased in fibroatheromas, TCFA, and plaque rupture. These data affirm that calcium burden is heaviest in stable plaques compared with the unstable plaques and show an inverse relationship with necrotic core area.

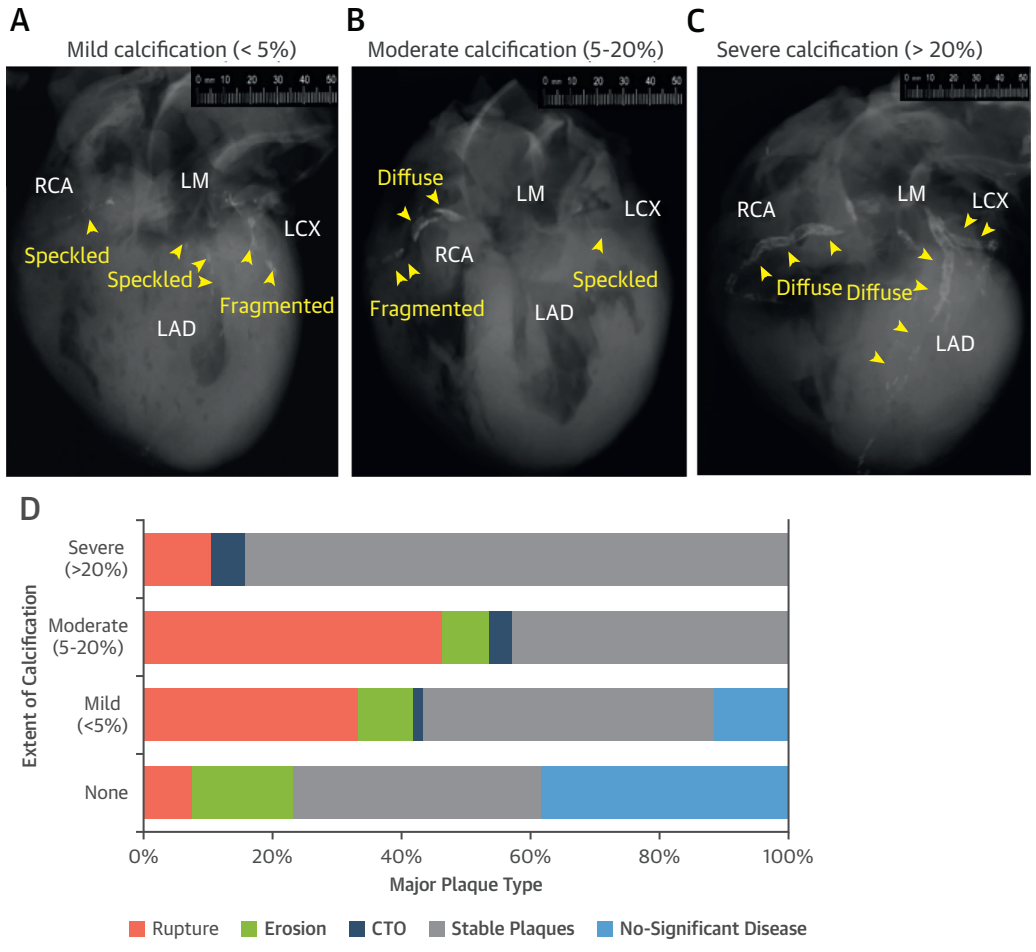
Another aspect worth mentioning is microcalcification within TCFA. Microcalcification is known to be present in thin cap, which is not visible by current in vivo imaging (5,6,43,44). The pioneering work of Vengrenyuk et al. (5) attributed cap rupture to microcalcification within the cap (6). It is thought that microcalcifications act as local tissue stress concentrators within the fibrous cap predisposing towards rupture (44). Although we agree that some ruptures are associated with cap microcalcification, this is not a uniform finding in plaque ruptures.

We have also assessed overall calcification level in whole heart radiographs as none (0%), mild (<5%), moderate (>5% to ≤20%), and severe (>20%), similar to the coronary calcium scoring pattern. Major plaque types per patient were selected based on the cause of death and stratified by level of calcification as detected by radiography (**Figure 5**). Rupture has predominantly mild to moderate calcification with the least calcification seen in erosions. However, the proportion of stable plaque (PIT, TCFA, and fibrocalcific plaque) was similar in none, mild, and moderate calcification groups, but greatest in the severe calcification group. In another autopsy study (45) of 510 coronary artery segments from 17 acute myocardial infarction (AMI) deaths, calcification was compared with 450 coronary segments from 15 age-matched noncardiac deaths. Calcification involved more coronary artery segments in AMI (47%) versus controls (25%), with higher calcification area in AMI patients. The degree of calcification in TCFA and ruptured plaques was significantly less than the stable, including PIT, TCFA, and fibrocalcific plaques. The extent of calcification correlated inversely with fibrous cap inflammation, but calcification was not identified as an independent determinant of unstable plaques in multivariate analysis. These data suggested that CAC score evaluation might be useful to detect generic risk of adverse coronary events in a population (because its presence suggests atherosclerosis), but it is not useful specifically to prospectively identify a culprit lesion. The relationship between calcification and plaque stability seems to differ according to age (46). Unstable lesions including plaque rupture or TCFA in subjects in their 4th decade showed higher calcification area than stable lesions but were not different during the 5th and 6th decades of life. By the 7th decade, greater calcification was observed in the stable plaques (47). Therefore, CAC could be more of a determinant of plaque instability in younger individuals.

PROGRESSION IN RESPONSE TO PHARMACOLOGIC INTERVENTION

High intensive statin therapy has been shown to be associated with plaque volume reduction by serial IVUS imaging studies (48–50). Puri et al. (51) assessed calcification as well as plaque volume by IVUS in patients receiving high-intensity statin therapy (HIST) ($n = 1,545$, defined as atorvastatin 80 mg or rosuvastatin 40 mg), low-intensity statin therapy (LIST) ($n = 1,726$, defined as atorvastatin <40 mg or rosuvastatin <20 mg, simvastatin <40 mg, pravastatin <80 mg, lovastatin <20 mg, and fluvastatin <40 mg), and no-statin therapy ($n = 224$) (51). HIST was associated with percent atheroma volume regression from baseline (−0.6%), whereas both LIST and no-statin therapy were associated with percent atheroma volume progression (+0.8%, +1.0%; $p < 0.001$ for all). Calcium was assessed by calcium indices (= [total number of analyzed frame with any calcium/total number of analyzed frames] × [maximal arc of calcium/4]). Intriguingly, significant increases in calcium indices from baseline were noted across all groups (median for HIST, +0.044; LIST, +0.038; no-statin, +0.020; $p < 0.001$ for all), which could relate to statin therapy ($p = 0.03$ for LIST vs. no-statin; $p = 0.007$ for HIST vs. no-statin; $p = 0.18$ for HIST vs. LIST). In other studies (52–54), statins have also been shown to be a risk factor for calcium progression. On the other hand, some CT studies showed conflicting

FIGURE 5 Extent of Calcification by Plaque Type



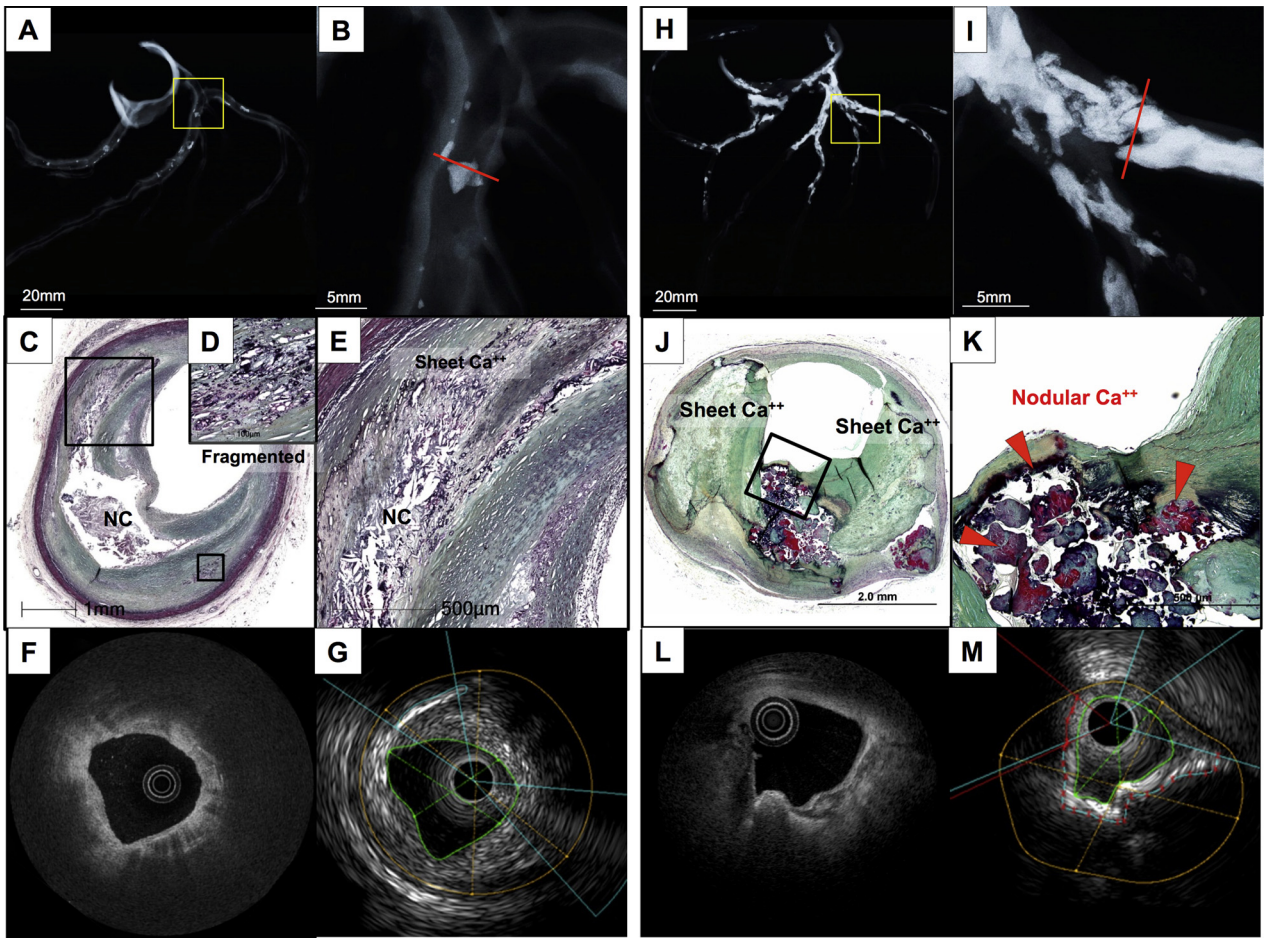
(A to C) Representative images showing different degrees in the extension of CAC as visual assessment in whole heart radiographs. **(D)** The distribution of major plaque types per patient. Stable plaque include PIT, TCFA, and fibrocalcific plaque with >75% luminal narrowing. Nonsignificant disease is defined as <75% luminal narrowing without thrombus. The data (n = 120) are obtained and reproduced with permission from Yahagi et al. (16). CAC = coronary artery calcification; CTO = chronic total occlusion, LAD = left anterior descending artery; LCX = left circumflex artery; LM = left main trunk; RCA = right coronary artery; other abbreviations as in Figures 1 and 2.

data regarding the effect of statin therapies on coronary calcium burden (55-57). The differences in patient background, the amount of agent, and study duration are all factors that might explain these conflicting results. Although both IVUS and CT have limitations in detecting calcium precisely and basic molecular mechanisms by which statins enhance plaque calcification are still under investigation, we do believe that statins may increase coronary calcification perhaps through calcification of the necrotic core which would tend to stabilize these plaques and reduce coronary events. Consequently, the CAC score calculated by CT might not be as precise in prognosis once plaque-altering treatment such as statins are initiated. This area of research deserves further attention.

INTRAVASCULAR IMAGING AND CALCIFICATION

Intravascular imaging using IVUS or optical coherence tomography (OCT) are also able to assess CAC (58,59). OCT or optical frequency domain imaging (OFDI) can detect sheets of calcification as indicated by signal poor dropout with sharp borderline. OCT-detected calcium can be confused with lipid or necrotic core,

FIGURE 6 Histologic, Radiographic, OFDI, and IVUS Appearance of Fragmented, Sheet, and Nodular Calcification

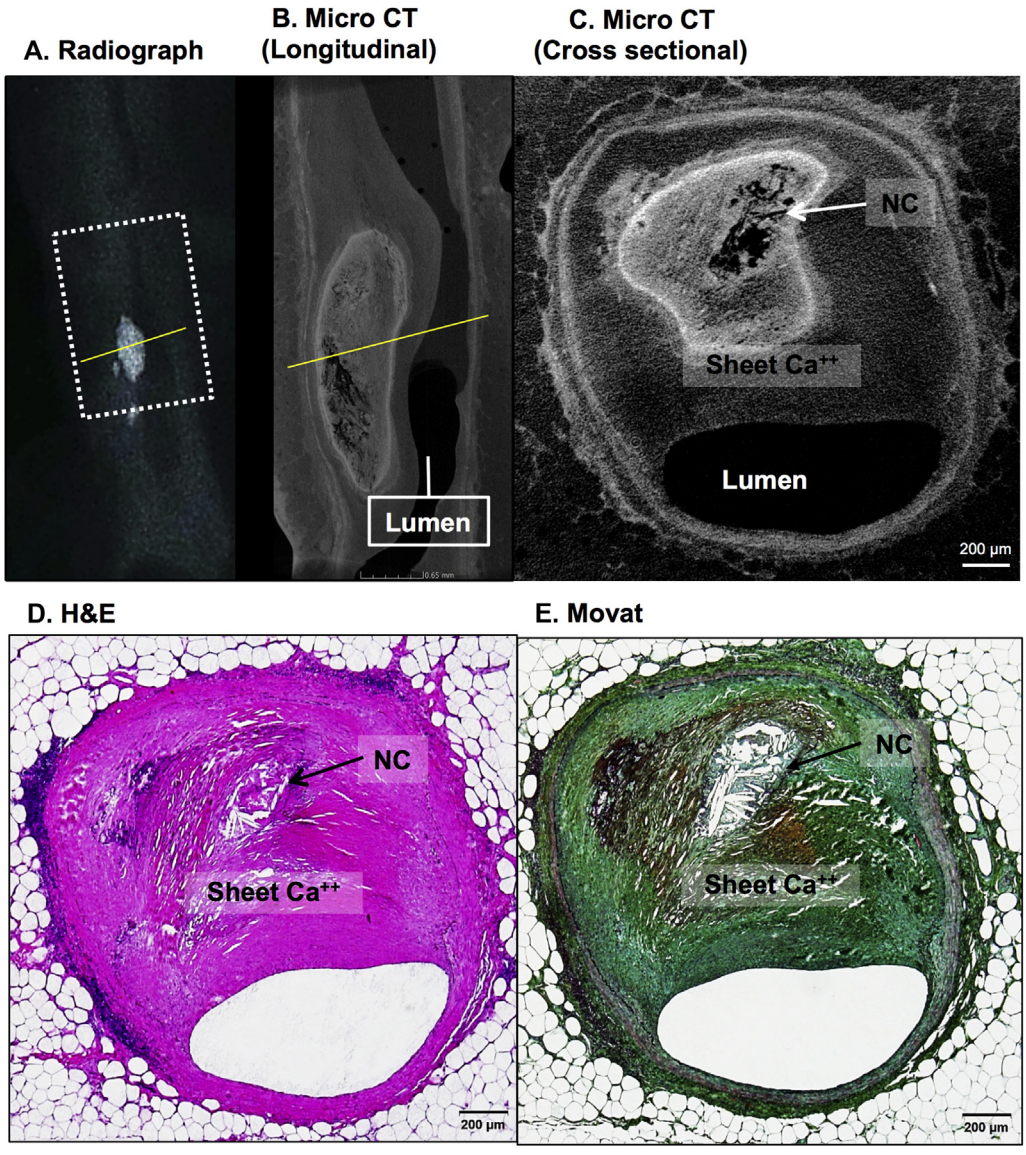


(A to G) Images from a 38-year-old male who died suddenly. **A** (low power) and **B** (high power) are radiographs of the epicardial coronary artery after removal from the heart showing speckled to fragmented type of calcification in proximal left anterior descending. Corresponding histologic images in **C (red line, B)** shows fibroatheroma with fragmented **(D)** and sheet **(E)** of calcium. OFDI performed in the same artery shows signal attenuation associated with lipid tissue (4 to 9 o'clock) **(F)**, whereas it is less apparent by IVUS **(G)**. IVUS shows presence of calcium **(G)** with acoustic shadow (10 to 12 o'clock), whereas it is less apparent by OFDI **(F)** due to necrotic core within calcium **(E)**. **(H to M)** Images from a 67-year-old male who died suddenly. **H** (low power) and **I** (high power) are radiographs showing diffuse calcification in proximal left circumflex. Corresponding histologic images in **J (red line, I)** and **K** (high power) show fibrocalcific plaque with nodular calcification. OFDI in **L** shows discreet surface border of sheet calcification (2 to 5 o'clock and 7 to 10 o'clock) and the calcified nodule mimics red thrombus appearance (5 to 7 o'clock). IVUS in **L** shows wide angle of calcification with acoustic shadowing (3 to 9 o'clock). **(J to M)** Images were obtained and reproduced with permission from Nakano et al. (58). IVUS = intravascular ultrasonography; OFDI = optical frequency domain imaging; other abbreviations as in Figure 1.

although the signal-poor regions of calcium are sharply delineated whereas the signal-poor regions of lipid or a necrotic core have poorly defined or diffuse borders (Figure 5). Although little information is available about different patterns of calcification, it seems that sheet calcification is what is most likely seen by this technology (60,61). The appearance of nodular calcification is very different from sheet calcification (Figure 6) and shows heterogeneous signal with high attenuation presumably due to fibrin interposed between nodules (60,61). When the nodule protrudes, which we call calcified nodule, the lumen surface appears irregular (60).

The IVUS appearance of calcium is echo dense (hyperechoic) plaque that is brighter than the surrounding reference tissue (Figure 6). However, it is limited by acoustic shadow cast by the calcified plaque (60,62). In an in vitro study examining IVUS

FIGURE 7 Micro CT of Fibroatheroma With Calcification

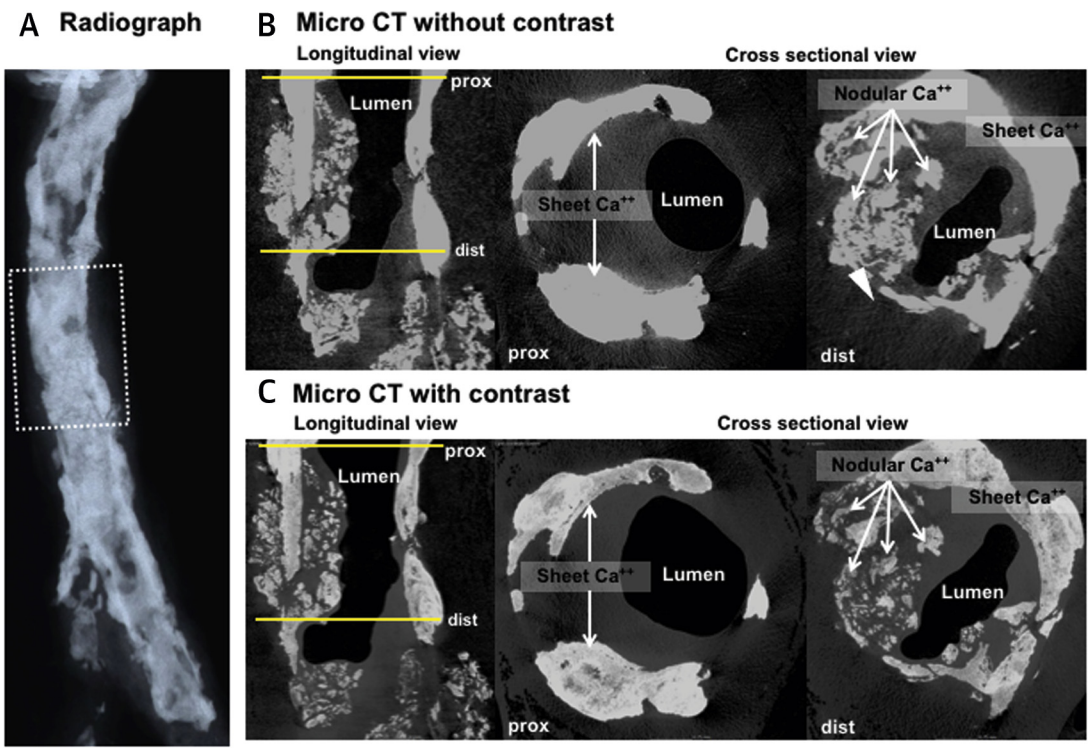


(A and B) Radiograph and micro CT lesions from a distal LAD in longitudinal view. A fragment of calcium shown by radiography **(A)** can be seen by micro CT **(B)** that has been stained using potassium iodine. The calcified area shows as bright image while the necrotic core within the site of calcification appears as black void space. **(C)** Micro CT cross-section. Following decalcification, histological sections **(D and E)** show a fibroatheroma with calcification. **Yellow lines in A and B** indicate the location of cross-section in **C to E**. CT = computed tomography; other abbreviations as in Figures 1 and 5.

imaging of human coronary artery explants, IVUS did not detect calcium in 14.8% of plaques containing histopathologic calcium. Reasons for this were deep calcium hidden behind large necrotic cores that produced echo attenuation, and the presence of microcalcific deposits. Nodular calcification can also be detected by using IVUS. Evaluation of the radial (arc) and longitudinal (length) extent is feasible by IVUS examination, but the assessment of thickness (depth) is not (40,41,60).

Assessing plaque progression or regression using gray scale IVUS or OCT is increasingly being used in clinical trials. Even though there are limitations of calcium assessment using these tools as stated above,

FIGURE 8 Sheet and Nodular Calcification by Micro CT



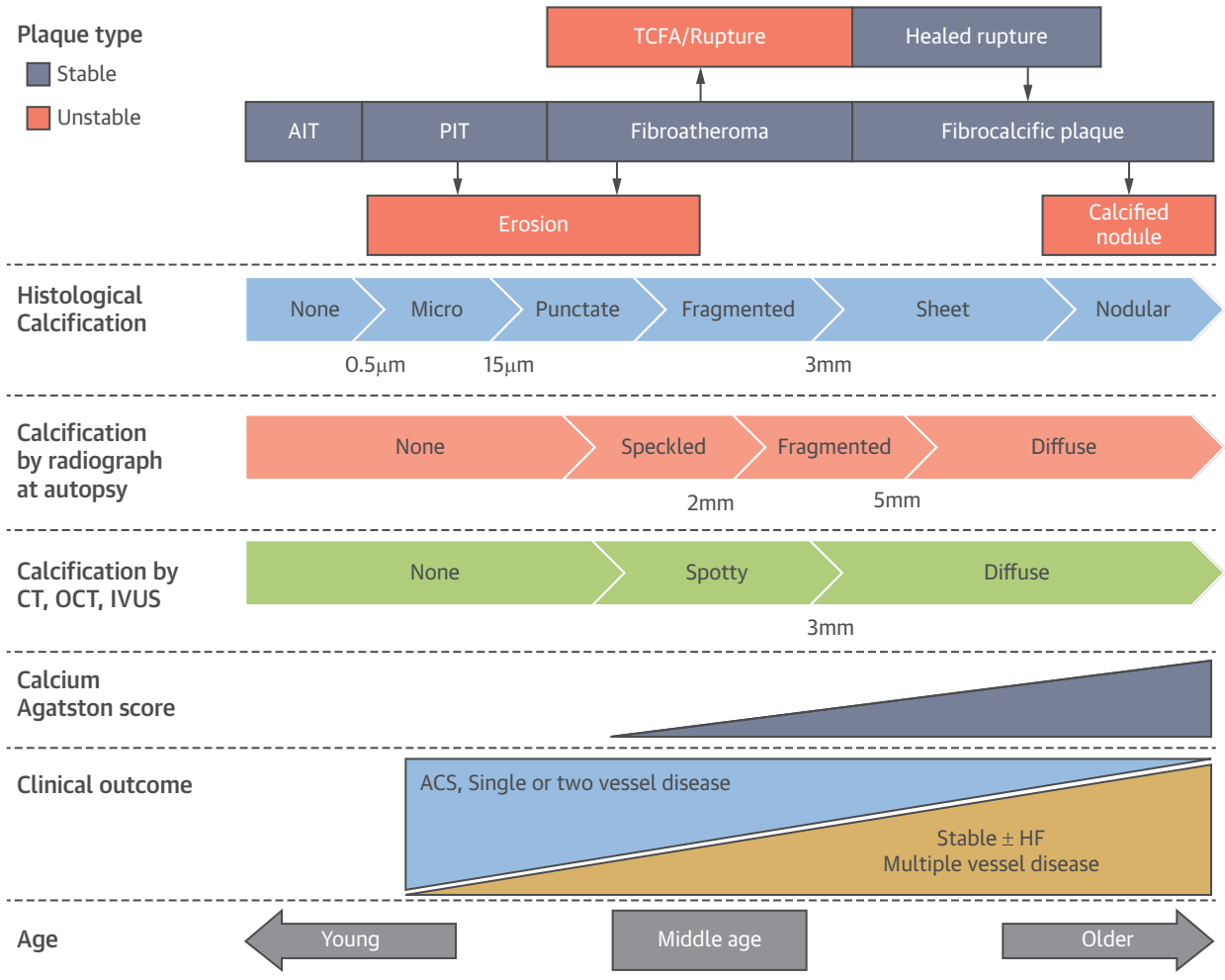
(A to C) Images of distal right coronary artery obtained from 55 year-old male who died suddenly during hemodialysis. Radiography **(A)**, and micro CT images **(B and C)** were obtained with and without contrast medium (10% MD-76 contrast, Mallinckrodt Inc., Missouri). The image in **A** shows extensive diffuse calcification, and the boxed area shows matched micro CT **(B and C)**. Sheet and nodular calcification are observed in both **B and C** but is more distinct after contrast staining. Abbreviations as in Figures 1, 3, and 7.

REVERSAL (Reversal of Atherosclerosis With Aggressive Lipid Lowering) and CAMELOT (Comparison of Amlodipine Versus Enalapril to Limit Occurrences of Thrombosis) studies reported that plaques with more calcium were more resistant to change than plaques with less calcium that were likely to progress or regress in response to pharmacologic interventions (40). On the other hand, the SATURN (Study of Coronary Atheroma by Intravascular Ultrasound: Effect of Rosuvastatin Versus Atorvastatin) trial showed an increase in dense calcium volume in 71 patients treated with high-dose rosuvastatin or atorvastatin for 24 months (63). Two other studies using IVUS with radiofrequency analyses (using virtual histology IVUS) showed that nontreated, fibrocalcific lesions did not progress during the 12 months observation period and were associated with lack of adverse events during 3-year follow-up (64,65). However, a recent meta-analysis and systematic review of the impact of statin therapy on coronary plaque composition as detected by virtual histology IIVUS suggested a significant increase in dense calcium only in patients taking statins (54). The discrepancy in the result of these trials may be caused by differences in patient backgrounds, amount and duration of pharmacological interventions, as well as difficulty in assessment of calcium in IVUS. Moreover, the limited resolution of IVUS for detection of changes in calcium makes it less than ideal as a tool to monitor calcium progression or regression.

CT AND CALCIFICATION

CT is the most important noninvasive tool to detect CAC. Calcium scoring, mainly the Agatston score, has been used to predict relative future risk of cardiovascular events in both asymptomatic and symptomatic persons (66). The Agatston score is a summed score of all calcified lesions that considers total calcified area and maximum density of calcification (>130 Hounsfield units [HU]). Although other methods of scoring have been used, the Agatston

CENTRAL ILLUSTRATION **Conceptual Diagram of Coronary Artery Calcification by Plaque Type (Stable and Unstable), Histology, Radiographic Noninvasive (CT, Agatston Score) and Invasive (OCT, IVUS) Assessment With Clinical Outcome Based on Age of Patients**



Mori, H. et al. J Am Coll Cardiol Img. 2018;11(1):127–42.

Diagram showing the theoretical relationship between plaque type (stable **[blue]** and unstable **[pink]**), histological pattern of calcification, radiographic appearance of calcification at autopsy, calcification identified by clinical imaging modalities (CT, OCT, and IVUS), calcium (Agatston) score, clinical outcome, and age of the patients at time of presentation are intertwined. Overall acute coronary syndromes, (i.e., acute myocardial infarction, unstable angina, and sudden coronary death with thrombi) have less calcification which is mainly fragmented as compared to individuals with stable angina with or without HMI who tend to show diffuse sheet calcification.
ACS = acute coronary syndrome; AIT = adaptive intimal thickening; CT = computed tomography; HF = heart failure; HMI = healed myocardial infarction; IVUS = intravascular ultrasound; OCT = optical coherence tomography; PIT = pathologic intimal thickening; TCFA = thin-cap fibroatheroma.

score is the gold standard because of its simplicity. The link between CAC and cardiovascular events occurs in a step-wise fashion and can be used to stratify risk for future coronary events. A low CAC score is also associated with decrease in overall and cancer-specific mortality. Thus, guidelines from around the world recommended CAC assessment to improve clinical risk prediction in appropriately selected asymptomatic individuals, especially those with an intermediate Framingham Risk Score of 5% to 20% (67).

It has been shown that presence of minimal CAC (i.e., CAC score 1 to 10) increases the risk of experiencing a coronary heart disease (CHD) event by 3-fold as compared with the absence of CAC. The hazard ratio for all-cause mortality was 1.99 (33). Moreover,

progression of CAC adds additional further predictive value and closely correlates with advancement of cardiovascular risk (68). Also, it has been shown that presence of diabetes increases cardiovascular disease risk; therefore, it may be prudent to add the CAC score to the mix of risk factors for predicting future events (69). The disconnect between calcium as a marker of stable fibrocalcific plaque and overall cardiovascular risk can be explained by the fact that higher CAC scores correlate with overall plaque burden. Patients with very high CAC scores have extensive coronary plaque burden with high likelihood of having stress-induced ischemia. Separate from the easily discovered heavily calcified plaque as detected by CT, the roles of noncalcified plaque and mixed plaques in determining future events should not be ignored.

A recent clinical CT study examined the predictive value of both calcium density (standard CAC Agatston score) and plaque calcium density. Although CAC was positively and independently associated with CHD and cardiovascular risk, at any level of CAC volume, CAC density was inversely and significantly associated with both CHD and cardiovascular risk (3). Similarly, the number of calcified coronary lesions and its pattern (diffuse vs. concentrated) enhanced risk prediction. These data suggest that further refinement of CAC scores may be warranted (66).

The emergence of multidetector CT has also spurred interest in noninvasive detection of vulnerable plaques (70). The characteristics of vulnerable coronary plaque are previously reported as positive vascular remodeling, low plaque density (<30 HU), napkin-ring sign, and spotty calcification (42,71). Although the presence of microcalcification strongly correlates with plaque instability, the present resolution of CT is still insufficient to detect its presence.

Currently, we have tried to identify plaque types more precisely using high-resolution micro CT (XTH 225 ST PE1621 EHS system, Nikon, Tokyo, Japan) on human coronary arteries obtained at autopsy. We have observed greater details of the plaque by micro CT imaging. Contrast with iodine gives greater clarity of the soft tissue and calcium. **Figure 7** shows micro CT images of calcified necrotic core showing a good correlation with histology. Not only cholesterol clefts but also outline of adventitial adipocytes was visible. **Figure 8** shows micro CT images of a heavy calcified coronary artery. Micro CT could clearly distinguish nodular calcification from sheet calcification, which is not possible with radiography alone. It goes without saying that getting these higher resolution images is much easier at the autopsy setting than in living patients. We were able to see the lumen, fragmented, sheet, and nodular calcification as well as necrotic core including cholesterol clefts and fibrous cap, which cannot be appreciated by other methods such as CT or radiography. Clinically, if image quality could be improved to this level, our understanding of atherosclerosis would be greatly enhanced; such advancement might improve diagnostic accuracy for the stratification of high-risk patients in daily clinical practice.

CLINICAL PERSPECTIVE

CAC is an important facet of CAD and a marker of overall disease burden. Early detection of CAC in younger subjects has important prognostic impact in terms of predicting future CHD risk (72). However, on a per patient basis, we cannot reliably predict who will have an event and when and where these will occur. When it comes to older patients, the proportion of fibrocalcific plaque and healed plaque rupture is higher, giving such plaques a higher calcium score. Some of these plaques may remain stable, and/or may develop severe stenosis without thrombosis, or go on to form calcified nodule resulting in thrombosis (**Central Illustration**). Using calcium to predict risk of future events on a per patient basis is not straightforward. As mentioned above, in the MESA study, CAC volume was associated with increased cardiovascular risk, although at any level of CAC volume, CAC density was inversely associated with the risk (3). It is possible that the effect of plaque stabilization by statins might be partially explained by enhancement of calcification in plaque necrotic core, although the basic mechanism is still unclear. Further investigation is required. From a pathological point of view, just looking at CAC score is not enough to understand the complexity of coronary disease, although it is very practical clinically. We believe presence of calcium (small, fragmented, spotty) is a better predictor of unstable plaque; however, heavy calcium (diffuse, fibrocalcific plaques, sheet of calcium) is a better predictor of stable plaque. To obtain a more accurate prediction, a new CAC scoring system based on higher resolution CT imaging than is currently available is warranted. Particular focus should be directed towards collecting information regarding early calcification consistent with higher risk for unstable coronary syndromes versus those with more diffuse calcification seen more frequently in stable disease.

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