

## REVIEW ARTICLE

# Pathology of Coronary Atherosclerotic Plaques and Mechanisms of Plaque Disruption

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## Abstract

Coronary atherosclerosis with acute thrombosis is the most common cause of sudden cardiac death. Because the disruption of coronary atherosclerotic plaques triggers acute thrombosis, it is important to identify disruption-prone plaques with imaging modalities. The pathology of “plaque disruption” includes three distinct morphologic entities: plaque rupture, plaque erosion, and calcified nodule. Currently, invasive imaging modalities such as intravascular ultrasound and intravascular optical coherence tomography can identify rupture-prone plaques, or thin-cap fibroatheromas, and calcified nodules, and unveiled that asymptomatic, multiple-plaque ruptures are a frequent complication in patients with coronary atherothrombosis. However, they cannot identify erosion-prone plaques. This article describes the morphological characteristics of coronary plaque disruptions and their possible mechanisms. In addition, the plaque imaging by nuclear medicine is discussed from a pathological viewpoint.

**Keywords:** Atherothrombosis, Coronary artery, Pathology, Plaque thrombogenicity

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Coronary atherosclerosis with acute thrombosis is the most common cause of sudden cardiac death. The disruption of atherosclerotic plaques triggers the development of acute thrombosis. Three distinct morphologic entities have been described as “plaque disruption:” plaque rupture, plaque erosion, and calcified nodule (1). For disruptions in sudden coronary death cases, 65% are plaque ruptures, 30% are plaque erosions, and 5% are calcified nodules (1). In contrast, asymptomatic coronary plaque disruption with thrombosis was reported in autopsy cases, and was more frequently identified with current clinical imaging modalities (2,3). Studies have demonstrated that the size of the thrombus is critical in determining whether disrupted plaques are symptomatic or asymptomatic. Although numerous studies have focused on the mechanisms of plaque development and instability, little is known about what characteristics of plaques modulate larger thrombus formation, or the exact mechanisms of plaque erosion and calcified nodule rupture.

## Plaque rupture

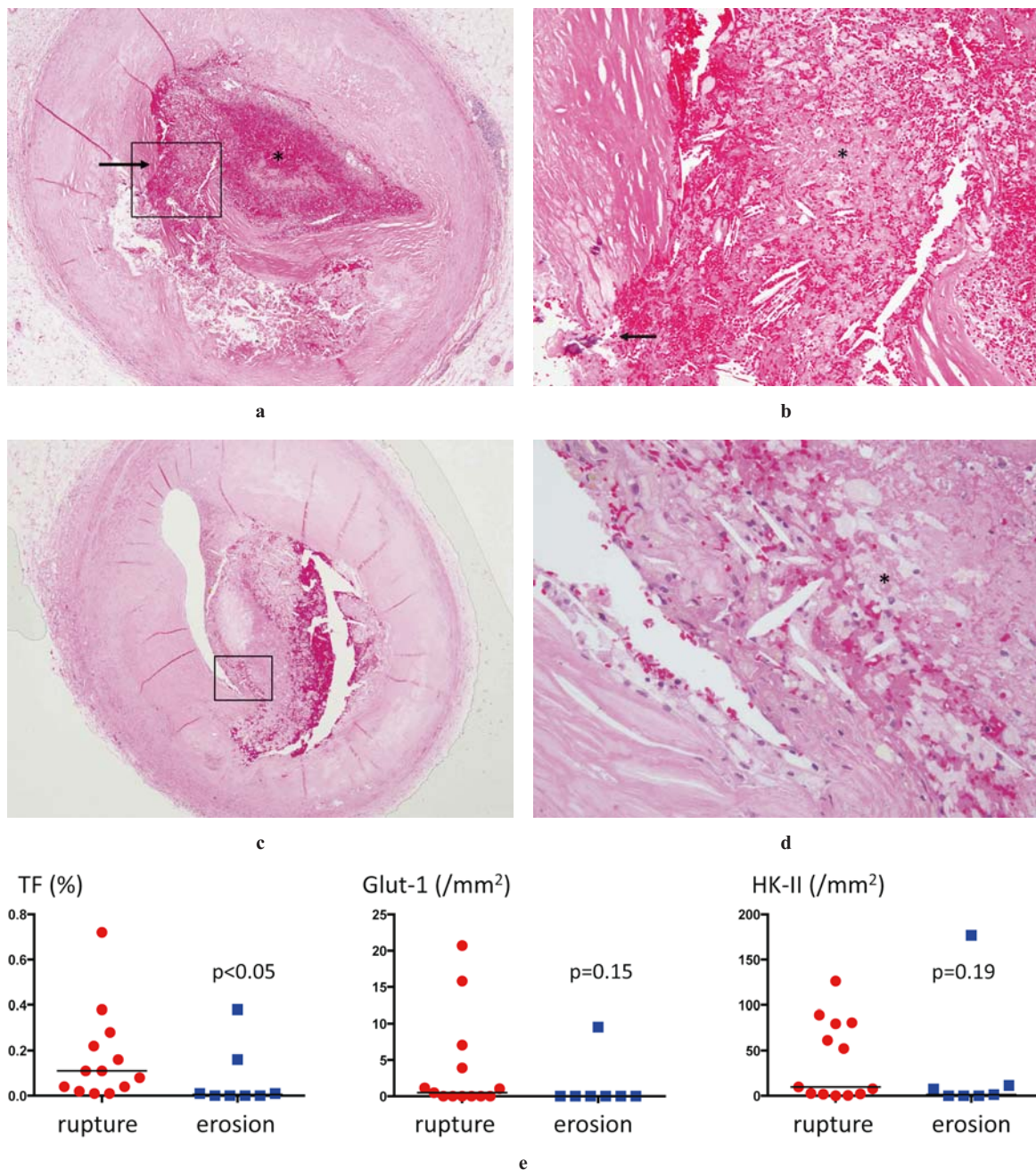
A plaque rupture is characterized by a luminal thrombus overlying a lipid-rich fibroatheroma with an interrupted thin fibrous cap (Fig. 1a and b). Plaque rupture allows a thrombogenic, necrotized (lipid) core to be exposed to platelets and coagulation factors, resulting in platelet-fibrin thrombus formation (4). Plaque rupture is most likely to occur in plaques with thin fibrous caps (usually  $<65\ \mu\text{m}$  thick), large necrotized cores, abundant macrophages and lymphocytes (4). Thus, a plaque with a thin fibrous cap is thought to be a precursor to plaque rupture, and is referred to as a thin-cap fibroatheroma (5) (Fig. 1c and d). Clinical studies have confirmed that the presence of thin-cap fibroatheromas is an independent risk factor for future cardiovascular events (6). These studies suggest that thin-cap fibroatheromas have rupture-prone plaque morphology. Current imaging modalities, including computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), intravascular ultrasound (IVUS), intravascular optical coherence tomography (OCT), and intravascular endoscopy are able

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**Fig. 1** Representative histological images of a ruptured coronary plaque and a thin-cap fibroatheroma and expression of thrombotic and metabolic factors in a patient with acute myocardial infarction.

- a:** Coronary atherosclerotic plaque of the culprit lesion, showing disruption of the fibrous cap (arrow) and occlusive thrombus formation (asterisk).
- b:** High magnification image at the disruption site (square in a) showing protrusion of thrombogenic necrotized tissue (asterisk), composed of necrotized tissue, foamy macrophages, and cholesterol clefts, into the coronary lumen. There are foci of microcalcification (arrow).
- c:** Coronary atherosclerotic plaque of the non-culprit lesion, showing thinning of the fibrous cap and intraplaque hemorrhage.
- d:** High magnification image of the plaque surface (square in c), showing thinning of fibrous tissue, infiltration by foamy macrophages, and close localization of the necrotized core (asterisk) to the lumen.
- e:** Expression of tissue factor, glucose transporter-1, and hexokinase-II in ruptured and eroded plaques in patients with acute myocardial infarction.

to visualize several high-risk plaque morphologies.

Though the likelihood is increased, plaque disruption does not always result in complete thrombotic occlusion with subsequent acute symptomatic events. In autopsies, various stages of healing plaque disruptions are found in cases with

and without cardiovascular diseases. Our autopsy study found coronary thrombi in 16% of non-cardiac deaths; most thrombi had developed on eroded plaques, and were too small to occlude the coronary lumens (2). Compared with plaque-induced acute myocardial infarction (AMI), healing plaques

showed smaller necrotized cores, thicker fibrous caps, and modest luminal narrowing (2). Recent clinical studies have shown that asymptomatic, multiple-plaque ruptures are a frequent complication in patients with coronary atherothrombosis (3, 7). Patients with non-culprit plaque ruptures had a higher prevalence of OCT-defined thin-cap fibroatheromas. Vergallo et al. recently reported that non-culprit lesions with plaque ruptures showed a smaller lipid burden, thicker fibrous caps, small plaque cavities, larger minimum lumen areas, and less thrombi, compared with culprit lesions with plaque ruptures (3). Autopsy and clinical data suggest that large thrombus formation after plaque disruption is a critical step in the development of AMI.

### Coronary plaque thrombogenicity

We studied the biological determinants of coronary thrombus size after plaque disruption in autopsy cases with AMI or asymptomatic plaque disruption (8). To do this, we focused on thrombogenic and metabolic factors. Thrombus size, length of plaque disruption, expression of tissue factor (TF), a coagulation initiator, and hexokinase (HK)-II, a glycolysis enzyme, were significantly larger in coronary plaques of AMI cases than in asymptomatic cases. Thrombus size was positively correlated with length of plaque disruption, TF, and HK-II expression. Our results suggest a possible relationship between thrombogenicity and glycolysis activity in plaques. In our rabbit atherothrombosis model, arterial  $^{18}\text{F}$ -fluorodeoxyglucose (FDG) uptake reflected TF expression and thrombus formation (9). This evidence indicates that  $^{18}\text{F}$ -FDG uptake in atherosclerotic lesions is a novel marker of thrombogenicity as well as glycolysis activity. One clinical study reported that higher  $^{18}\text{F}$ -FDG uptake in plaques was associated with acute coronary thrombosis (10). However, the studies of human coronary arteries using  $^{18}\text{F}$ -FDG-PET are limited, due to high uptake of  $^{18}\text{F}$ -FDG in cardiac myocytes.

The underlying mechanisms linking arterial glucose uptake to vascular thrombogenicity remain unclear. Hypoxia is an important microenvironmental factor that influences the progression of atherosclerosis by inducing foam cell formation, necrotic core expansion, and plaque neovascularization. Reversal of hypoxia in atherosclerotic plaques by breathing hyperoxic carbogen gas reduced plaque hypoxia, necrotic core size, and apoptotic cell content and increased efferocytosis of apoptotic cells by macrophages in mice (11). In our study, hypoxic areas detected by pimonidazole immunostaining were mainly localized in the deep portion of lipid and macrophage-rich plaques, but not in normal arteries of rabbits (12). The hypoxic areas were positively correlated with plaque size, nuclear localization of TF expression, and fibrin located in the developed thrombus (12). Moreover, the nuclear localization of hypoxia-inducible factor (HIF)-1 $\alpha$  correlates with TF and

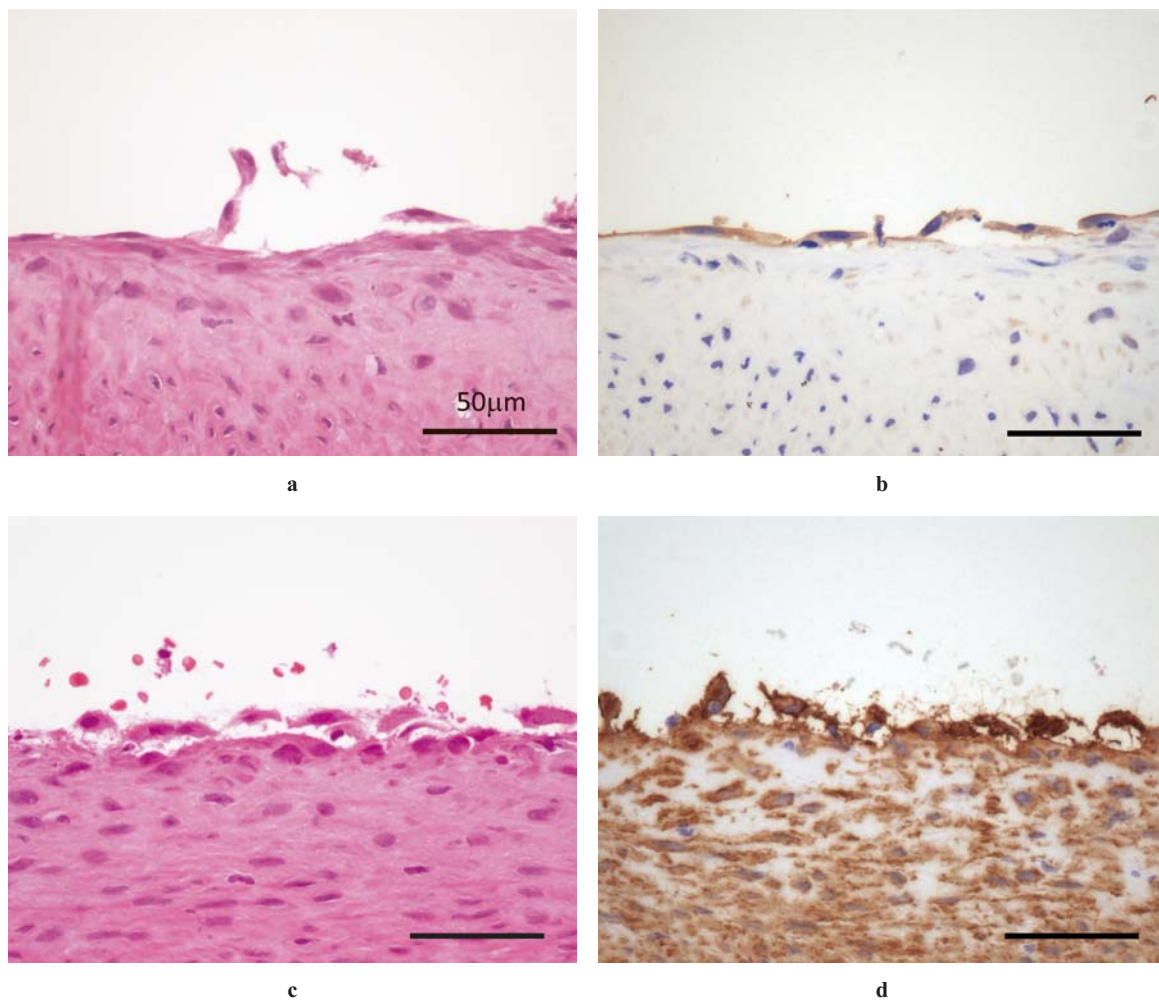
fibrin expression in human coronary atherosclerosis (12). These findings support the notion that hypoxic stimuli enhance thrombogenicity in symptomatic plaques. Because hypoxia enhances glucose uptake and affects glycolysis in cells involved in atherosclerosis (13), hypoxia may link glucose metabolism to thrombogenicity in atherosclerotic plaques. Several studies have visualized hypoxia in rabbit atherosclerotic lesions, using the hypoxia probes  $^{18}\text{F}$ -fluoromisonidazole and  $^{64}\text{Cu}$ -ATSM (14, 15). Therefore, non-invasive detection of vascular hypoxia may be a promising tool to assess thrombogenicity in coronary atherosclerotic plaques.

### Plaque erosion and possible mechanism

Eroded plaques are characterized by a denuded surface, luminal thrombus, and lack of a disrupted, fibrous cap (16). Patients with plaque erosion are younger, and are not predominantly males compared with those who have ruptured plaque. Angiography has shown that the vascular lumens of eroded plaques are less irregular and less narrow. The morphological features of eroded plaques include abundant smooth muscle cell (SMC)s, proteoglycan matrices, and disrupted surface endothelia. Eroded plaque contains relatively few macrophages and T cells compared with ruptured plaque (16). In contrast, another study showed that inflammatory cells are the dominant cell type at both plaque rupture and erosion sites (17). These findings suggest that eroded plaques are heterogeneous in nature, and that both inflammatory and non-inflammatory processes may contribute to the development of plaque erosion. To identify thrombogenic potential and glycolytic activity in ruptured and eroded plaques, we examined TF, glucose transporter (Glut)-1, and HK-II expression in 20 autopsy cases of AMI (13 ruptures, 7 erosions). TF expression in ruptured plaques was larger than that in eroded plaques. Glut-1 and HK-II expression in ruptured plaques tended to be more than that in eroded plaques, but not statistically significant (Fig. 1e). The results suggest abundant thrombogenic potential in ruptured plaque and heterogeneous glycolytic activity in symptomatic plaques.

Eroded plaques are characterized by superficial plaque injury, but the mechanisms of the superficial injury are poorly understood. Experimental aortic stenosis in normal aortas can induce acute endothelial changes or damage (18). Therefore, hemodynamic forces, generated by stenosis or vasoconstriction, are likely a crucial factor in inducing surface vascular damage and thrombosis. Acute vascular narrowing disrupted the blood flow in these arteries, inducing superficial erosive injury (detachment of endothelial cells and surface SMCs) of the SMC-rich plaque in rabbits (Fig. 2) (19). Subsequent vascular narrowing led to the development of mural thrombi, consisting of platelets and fibrin, as in human plaque erosion. A computational fluid simulation using the Reynolds-





**Fig. 2** Representative histological images of rabbit femoral artery 15 minutes after vascular narrowing.

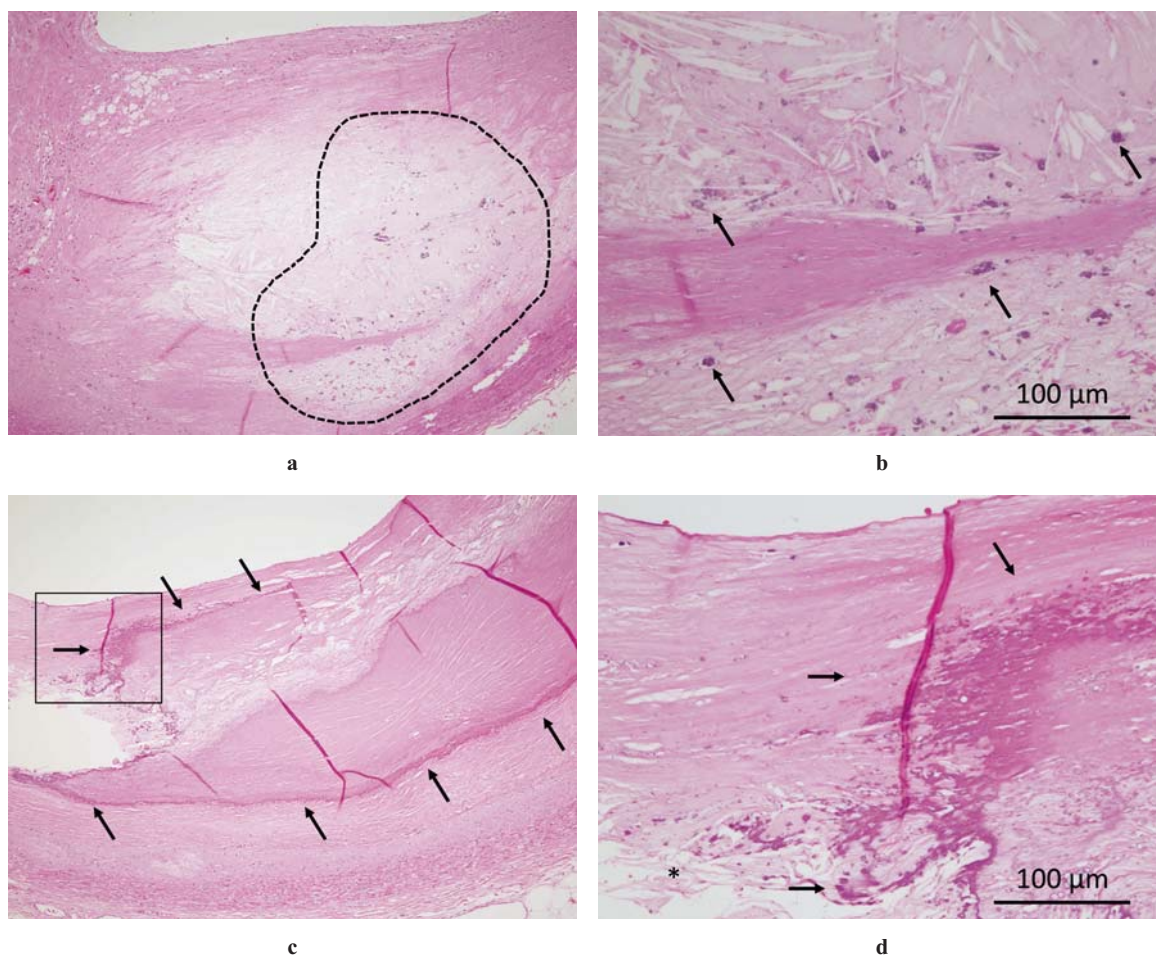
The longitudinal section of the rabbit femoral artery shows endothelial (a, b) and smooth muscle cell (c, d) detachments after vascular narrowing (hematoxylin-eosin stain). Immunohistochemistry for von Willebrand factor (b) and smooth muscle actin (d) highlights the endothelial and smooth muscle cell detachments (from ref. 18).

averaged Navier-Stokes model showed that the magnitude of wall shear stress, turbulent kinetic energy, and blood pressure gradients were significantly larger at eroded sites compared with non-eroded sites (20). Thus, disrupted blood flow can induce superficial erosive damage to SMC-rich neointima and subsequent thrombus formation. Clinical evidence supports the notion that coronary artery vasospasm is accompanied by erosive injury and thrombus formation. Percutaneous transluminal coronary angiography in patients with vasospastic angina showed intimal injuries (hemorrhage, flap, thrombus, or ulcer) in 4 out of 10 patients after spontaneous vasospasm or intracoronary acetylcholine-induced vasospasm (21). OCT-defined erosions and thrombi were observed in a quarter of coronary vasospastic sites in patients with vasospastic angina (22). These findings suggest a causal relationship between coronary vasospasm and erosive plaque injuries.

#### Calcified nodule

A calcified nodule is a distinct form of calcification in

atherosclerotic lesions that may rarely rupture, causing acute thrombosis. The prevalence of ruptured calcified nodules was 5% of acute thrombotic lesions in patients with sudden coronary death (1), and 8% of OCT-defined culprit lesions in patients with ACS (23). The ruptured calcified nodule is characterized by irregular blocks of calcified plaques surrounded by hemorrhage and fibrin. The underlying mechanisms of rupture of calcified nodules remain unknown. It is speculated that mechanical stress on the heavily calcified coronary arteries might fragment the plaque, resulting in smaller nodules surrounded by fibrin, which may cut through the plaque surface (24). In an intravascular IVUS study, the prevalence of calcified nodules in culprit and non-culprit coronary segments was 17% per artery and 30% per patient with ACS. Interestingly, the patients with calcified nodules in non-culprit coronary segments experienced fewer major adverse events during the 3 years of follow-up (25). Mechanisms of calcified nodule formation and its rupture in coronary arteries remain unknown. Therefore, further studies



**Fig. 3** Representative image of coronary atherosclerotic calcification.

- a:** There is a focus of microcalcification in the coronary fibroatheroma (inside of dash line).
- b:** High magnification image of the fibroatheroma, showing vesicular depositions in the necrotized core and beside cholesterol cleft, suggesting dead macrophages.
- c:** Coronary atherosclerotic plaque, showing plate-like macrocalcification in the acellular plaque. The arrows show the calcified border in the plaque.
- d:** High magnification image of the square in c, showing a border of macrocalcification (arrows). Vesicles of microcalcification are also present (asterisk).

are required to identify detailed morphological findings of symptomatic calcified nodule and mechanisms of calcified nodule formation.

### Coronary calcification

Calcification is a feature of atherosclerosis, and there are two types of atherosclerotic calcification. Minute calcified microvesicles in atherosclerotic lesions are called microcalcifications (Fig. 3a and b). Confluent calcified plates or sheets in advanced plaques are called macrocalcifications (Fig. 3c and d), which can be visualized by radiography, CT, and MRI. Macrocalcification is a reliable marker of coronary plaque burden, and an independent risk factor for adverse outcome (26). Interestingly, macrocalcification is not a reliable marker for plaque instability and acute coronary thrombosis. Microcalcification within the fibrous cap may predispose to rupture, due to substantial stress accumulation within the fibrous cap (27).

$^{18}\text{F}$ -sodium fluoride ( $^{18}\text{F}$ -NaF) is a positron emission tomography (PET) tracer that detects novel areas of bone formation and remodeling. Coronary  $^{18}\text{F}$ -NaF uptake was shown to be higher in patients with coronary atherosclerosis than in control subjects; however, uptake invariably colocalized with calcification (28).  $^{18}\text{F}$ -NaF uptake in aortic atherosclerosis was inversely correlated with aortic calcification on PET/CT (29). Patients with stable angina had plaques with focal  $^{18}\text{F}$ -NaF uptake that were associated with higher-risk features (positive remodeling and necrotic core) and microcalcification on IVUS than those without uptake. Marked  $^{18}\text{F}$ -NaF uptake occurred at the sites of all carotid plaque ruptures, and was associated with histological evidence of active calcification (tissue non-specific alkaline phosphatase and osteocalcin), macrophage infiltration, apoptosis, and necrosis (30). This evidence suggests that  $^{18}\text{F}$ -NaF uptake reflects the active phase of microcalcification, and is a novel marker for plaque instability.

## Conclusion

In plaque morphology, thin-cap fibroatheromas are likely rupture-prone. In addition to detection of thin-cap fibroatheromas, non-invasive detection of vascular hypoxia may be a promising tool to assess thrombogenicity in coronary atherosclerotic plaques. Although no distinct pathological features of erosion-prone plaques have been identified, acute luminal narrowing due to coronary vasospasm may induce superficial erosive injury. The clinical significance of calcified nodules is still controversial, and though intraplaque hemorrhages were not described in this article, they may contribute to necrotic core expansion and plaque instability.

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## Conflicts of interest

None.

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