Histopathology of Atherosclerosis Progression: What Imagers Need to Know

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Abbreviations

ACS	Acute coronary syndrome
AHA	American Heart Association
AMI	Acute myocardial infarction
CTO	Chronic total occlusion
CVD	Cardiovascular disease
Hb	Hemoglobin
HO-1	Hemoxygenase-1
Hp	Haptoglobin
HPR	Healed plaque rupture
ICAM-1	Intercellular adhesion molecule 1
MMP	Matrix metalloprotease
MPO	Myeloperoxidase
NO	Nitric oxide
PCI	Percutaneous coronary intervention
PIT	Pathologic intimal thickening
SCD	Sudden coronary death
SEM	Scanning electron microscopy
SMC	Smooth muscle cell
TCFA	Thin-cap fibroatheroma
TF	Tissue factor
VCAM-1	Vascular cell adhesion molecule 1

1 Introduction

Atheromatous coronary artery disease is the leading causes of death worldwide, constituting approximately 7,000,000 cases each year. Atherosclerotic plaque rupture with thrombosis is the pathologic mechanism responsible for the majority of acute myocardial infarction (AMI) and sudden coronary death (SCD). Insights into the mechanism of luminal thrombosis have been elucidated from the study of the diseased arterial wall, largely through detailed analysis of the underlying plaque morphologies observed in pathologic studies of sudden death victims. While formerly the emphasis has been on luminal narrowing, the interventionalists today must have an understanding of specific plaque composition to potentially identify rupture-prone arterial plaques. This understanding is critical, especially as new imaging modalities emerge, which are targeted at identifying vulnerable plaques.

In the early 1990s, the American Heart Association (AHA) proposed a classification scheme for coronary atherosclerosis progression by which atherosclerotic lesions were classified into six numerical categories categorized in relation to plaque rupture. In this classification scheme, plaque rupture was identified as the sole etiology of coronary thrombosis [1, 2]. Sudden luminal thrombosis however may arise from three different plaque morphologies: plaque rupture, erosion, and calcified nodule. Moreover, the AHA nomenclature failed to describe healing mechanisms, which contribute to severe luminal narrowing with or without acute plaque rupture. These silent plaque ruptures may also lead to chronic total occlusion (CTO), which is reported to occur in approximately 30 % of SCDs. These limitations led us to modify the AHA classification, forgoing the more complicated numeric categories in favor of a simpler descriptive classification. This classification system can easily be translated and utilized by new invasive and noninvasive imaging modalities to improve our predictive ability in patients that are at high risk of developing acute coronary syndromes (ACSs) [3]. Moreover, to improve the outcome of patients with ACS, it is essential to have a comprehensive understanding of pathological processes involved in the progression of atherosclerosis as discussed below.

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Table 2.1 Modified AHA consensus classification based on morphologic description

	Description	Thrombosis
Non-atherosclerotic intimal lesions		
Intimal thickening	Normal accumulation of smooth muscle cells (SMCs) in the intima in the absence of lipid or macrophage foam cells	Absent
Intimal xanthoma	Superficial accumulation of foam cells without a necrotic core or fibrous cap. Based on animal and human data, such lesions usually regress	Absent
Progressive atherosclerotic lesions		
Pathologic intimal thickening	SMC-rich plaque with proteoglycan matrix and focal accumulation of extracellular lipid	Absent
Fibrous cap atheroma	Early necrosis: focal macrophage infiltration into areas of lipid pools with an overlying fibrous cap Late necrosis: loss of matrix and extensive cellular debris with an overlying fibrous cap	Absent
Thin-cap fibroatheroma	A thin fibrous cap (<65 μm) infiltrated by macrophages and lymphocytes with rare or absence of SMCs and relatively large underlying necrotic core. Intraplaque hemorrhage/fibrin may be present	Absent
Lesions with acute thrombi		
Plaque rupture	Fibroatheroma with cap disruption; the luminal thrombus communicates with the underlying necrotic core	Occlusive or non-occlusive
Plaque erosion	Plaque composition, as above; no communication of the thrombus with necrotic core. Can occur on a plaque substrate of pathologic intimal thickening or fibroatheroma	Usually non-occlusive
Calcified nodule	Eruptive (shedding) of calcified nodule with an underlying fibrocalcific plaque with minimal or absence of necrosis	Usually non-occlusive
Lesions with healed thrombi		
Fibrotic (without calcification) Fibrocalcific (±necrotic core)	Collagen-rich plaque with significant luminal stenosis. Lesions may contain large areas of calcification with few inflammatory cells and absence of necrosis. These lesions may represent healed erosions or ruptures	Absent

(Modified from Virmani R et al (2000) Lessons from sudden coronary death: a comprehensive morphological classification scheme for atherosclerotic lesions. Arterioscler Thromb Vasc Biol 20:1262–1275)

2 Progression of Atherosclerotic Plaque

In the modified classification (Table 2.1, Figs. 2.1 and 2.2), the numeric AHA types I to IV are replaced by descriptive terminology: adaptive intimal thickening, intimal xanthoma (fatty streak), pathologic intimal thickening (PIT), and fibroatheroma. AHA type V and type VI lesions were discarded since they failed to account for the three different causes of thrombotic morphologies (rupture, erosion, and calcified nodule) and their relationship to healed plaque rupture (HPR) that is representative of stable angina. Since atherosclerosis is a dynamic process with a complicated pathogenesis, it is useful to review the stages of development of atherosclerosis. As the mechanistic underpinnings of the disease are better understood, however, the classification should be revised periodically and improved as new knowledge is gained.

2.1 Adaptive Intimal Thickening and Intimal Xanthoma (Fatty Streaks)

The earliest manifestation of vascular change is "adaptive intimal thickening" (AHA Type I), which consists of several layers of smooth muscle cells (SMCs) in an extracellular matrix with no or little inflammatory cell infiltration. Intimal thickening is observed in 35 % of neonates, and the intima/media ratio at birth is 0.1 increasing progressively to reach 0.3 by 2 years of life [4]. This change is considered adaptive (non-atherosclerotic) since the SMCs exhibit a very low proliferative activity and exhibit an anti-apoptotic phenotype [5]. Although the adaptive intima increases in thickness with aging, very rarely does it grow into a disease compromising blood flow. The change of shear stress is a trigger for abnormal responses in the endothelial lining [6] as well as changes secondarily induced in SMC phenotype [7]; however, the detailed mechanism remain elusive.

"Fatty streak" or "Intimal xanthoma" (AHA Type II) is a lesion that is not raised and is primarily composed of abundant foamy macrophages interspersed between SMCs. Although the AHA classification alludes to this entity as the earliest lesion of atherosclerosis, from our experience and reports of human and animal studies, the lesion is reversible at least in some locations [8, 9]. Some reports suggest that the modification of extracellular matrix is responsible for the progression of macrophage infiltration implying a role for biglycan and decorin proteoglycans [10], but the mechanism remains largely uncertain.

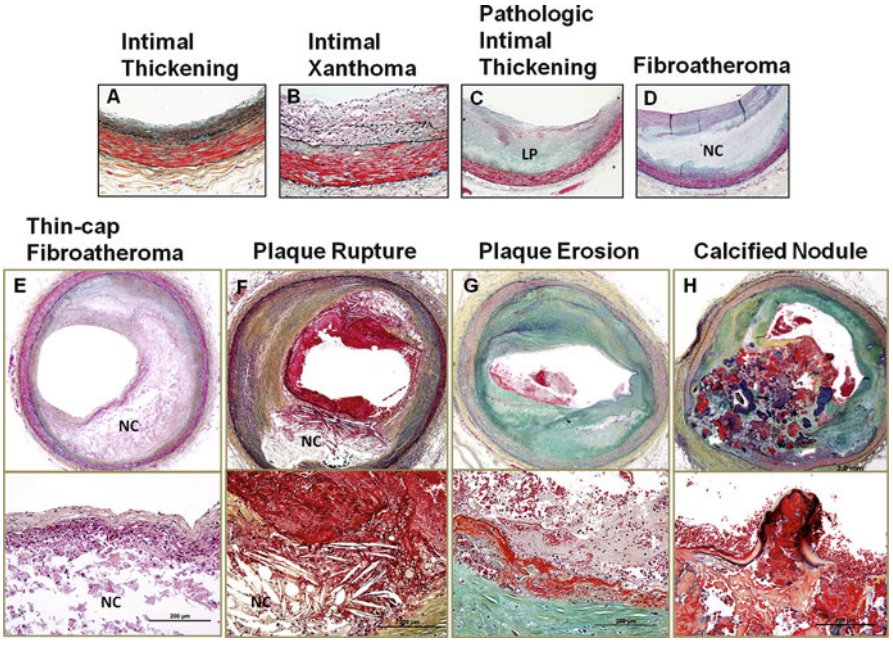

Fig. 2.1 Plaque atherosclerotic progression in human coronary arteries. These histologic images illustrate the various lesion morphologies of human coronary atherosclerosis. (**a**) Adaptive intimal thickening is present from birth and consists of a smooth muscle-rich intima. (**b**) Intimal xanthoma are predominantly foam cell-rich lesions that are found in the young, but known to regress in adults. (**c**) Pathologic intimal thickening (PIT) is the first of the progressive plaques marked by an acellular lipid pool rich in proteoglycan; inflammation in macrophages when present is typically confined to the most luminal aspect of this plaque. (**d**) Fibroatheroma (FA) are lesions with areas of necrosis characterized by cellular debris and cholesterol monohydrate with varying degrees of calcification or hemorrhage. (**e**) Thin-cap fibroatheroma (TCFA) or vulnerable plaques are recognized by their relatively large necrotic core and thin fibrous cap which is infiltrated by numerous macrophages. (**f**) Plaque rupture leads to exposure of necrotic contents to the blood flow, resulting in the triggering of the coagulation cascade. The luminal thrombus at the site of rupture is platelet rich (*white thrombus*). (**g**) Erosion is another entity that gives rise to coronary thrombosis. Erosions can occur on a substrate of PIT or FA. (**h**) Calcified nodules (a minor but viable mechanisms of thrombosis) depict eruptive fragments of calcium that protrude into the lumen causing a thrombotic event. *LP* lipid pool, *NC* necrotic core

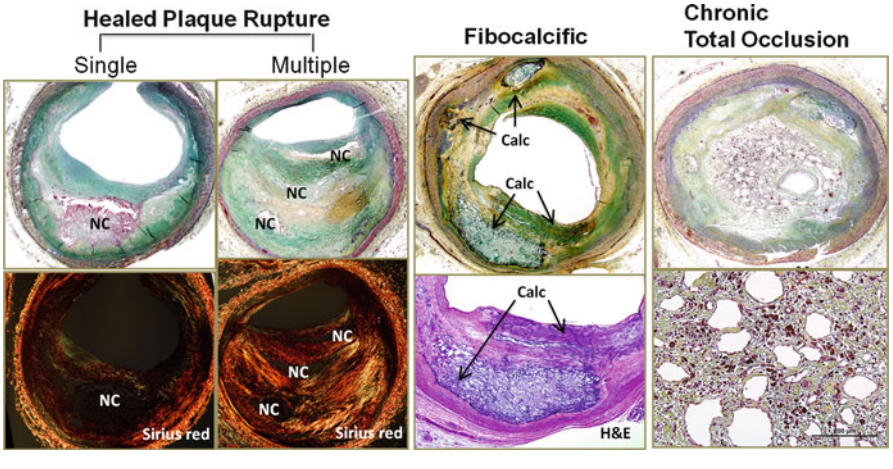

Fig. 2.2 Fate of atherosclerotic plaque. *Boxes* on the *left* show healed plaque ruptures where the newly formed fibrous cap is rich in proteoglycans and type III collagen (type III collagen appears *green* with Sirius red stain under polarized light) with interspersed smooth muscle cells. Repeated ruptures lead to a multilayered appearance with necrotic cores and overlying fibrous layers, resulting in severe luminal narrowing. Multiple healed plaque ruptures are thought responsible for progressive luminal narrowing. Fibrocalcific plaque (*middle column*) is thought to be a burnt-out lesion, which is associated with a calcified sheet of plaque matrix (*arrows*). *Boxes* on the *right* show histologic features of chronic total occlusion. The lumen is characterized by recanalized organized thrombus that is rich in proteoglycan matrix with presence of iron deposition and macrophages. *Calc* calcification, *NC* necrotic core

2.2 Pathologic Intimal Thickening

"Pathologic intimal thickening" (PIT, AHA Type III) is recognized as the earliest progressive (irreversible) lesion by most research groups. The lesion is characterized by layers of proliferating SMCs near the lumen and an underlying lipid pool present at the intimal medial border. The origin of lipid pool is not fully understood. The area of the lipid pool is rich in proteoglycan versican and hyaluronan as well as extracellular lipid deposits but is devoid of SMCs and macrophages. It has been demonstrated that there is an affinity of the lipid pool to retain plasma lipoprotein, which suggests that the accumulation of extracellular lipid is likely derived from the influx of plasma lipoproteins [11]. Williams et al. proposed a "response-to-retention" hypothesis: the retention of atherogenic lipoprotein associated with the extracellular matrix such as proteoglycan versican and hyaluronan is an initiating event in early atherogenesis [12]. Recent studies reinforce this hypothesis by demonstrating that structural changes in the glycosaminoglycan chain of proteoglycans are an initial proatherogenic step that promotes the binding and retention of lipoproteins [13]. An alternative hypothesis suggests that the membranes of apoptotic SMC may be an alternative source for lipid in PIT [14]. Apoptotic SMCs within lipid pools are recognized by membrane remnants (cages of basal lamina) and the presence of microcalcification representing calcified mitochondria [15]. However, the proof supporting this mechanism remains speculative.

Another important hallmark of PIT is the presence of varying degrees of foamy macrophage accumulation near the luminal aspect of the plaque (apart from the lipid pool) albeit this does not necessarily apply to all cases. Lesions demonstrating PIT with foamy macrophages are considered a more advanced stage of atherosclerosis as reported by Nakashima et al. in their systematic study of early coronary plaques [16]. We believe that macrophages invade the plaque from the luminal surface. Although the precise nature of focal macrophage accumulation in PIT is not fully elucidated, it is speculated that retention or modified lipoprotein along with activation of vascular adhesion molecules like VCAM-1 and ICAM-1 expressed by endothelial cells stimulates the recruitment of macrophages [17, 18]. In addition, lesions with PIT exhibit varying degrees of free cholesterol represented by empty fine crystalline structures in paraffin-embedded sections that accumulate within lipid pools. Although it is assumed that free cholesterol originates from dead foam cells, this is not a likely source in PIT as the majority of macrophages when present are confined to the more luminal aspect of the plaque.

2.3 Fibroatheroma

Fibroatheroma (AHA type IV) represents a further progressive stage of atherosclerotic disease and is histologically characterized by the presence of acellular necrotic cores, which are distinct from the lipid pools of PIT as they lack expression of hyaluronan and proteoglycan versican. Recognition of early macrophage infiltration into the lipid pools and cell death along with a substantial increase in free cholesterol and breakdown of extracellular matrix, which is presumably degraded by the matrix proteases released by macrophages, is classified as "early" necrotic cores. In the early phase of the necrotic core formation an efficient system of clearance of apoptotic bodies by macrophages is present, however, the system is soon overwhelmed and there is defective phagocytic clearance of apoptotic cells and this is thought to further contribute to the vicious circle of enlargement of necrotic core and plaque progression (Fig. 2.3) [19]. As total plaque burden increases, compensatory enlargement of the vessel, i.e., positive coronary arterial remodeling, occurs to preserve arterial lumen. According to Glagov, the lumen compromise only begins to occur when the luminal narrowing exceeds >40 % cross-sectional luminal narrowing [20]. We have further classified fibroatheromas into "early" and "late" based on the type of necrotic core observed. A necrotic core that is devoid of proteoglycan versican and hyaluronan or any collagen expression is termed "late" necrotic core but a thick fibrous cap fully contains the necrotic core. On the other hand "early" necrotic cores focally express the proteoglycan versican and hyaluronan, especially towards the media, but towards the lumen there is absence of matrix with macrophages infiltration.

2.4 Intraplaque Hemorrhage

It is not only apoptotic macrophages that contribute to the accumulation of free cholesterol but other source such as red blood cells may also contribute to the expansion of the necrotic core (Fig. 2.4). Studies from our SCD registry showed that hemorrhage into a necrotic core is commonly observed in cases of plaque rupture and late necrotic core. The membranes of red blood cells are enriched with lipid, which constitutes 40 % by weight, and are rich in free cholesterol content that exceeds that of all other cell membranes [21]. Excess membrane cholesterol of red blood cells can phase separate and form immiscible membrane domains consisting of pure cholesterol arranged in a tail-to-tail orientation favoring crystal formation [22]. The extent of accumulated erythrocytes incorporated into the plaque and abundant lipids, together with impaired phagocytic efficiency of macrophages to effectively clean up red blood cells and

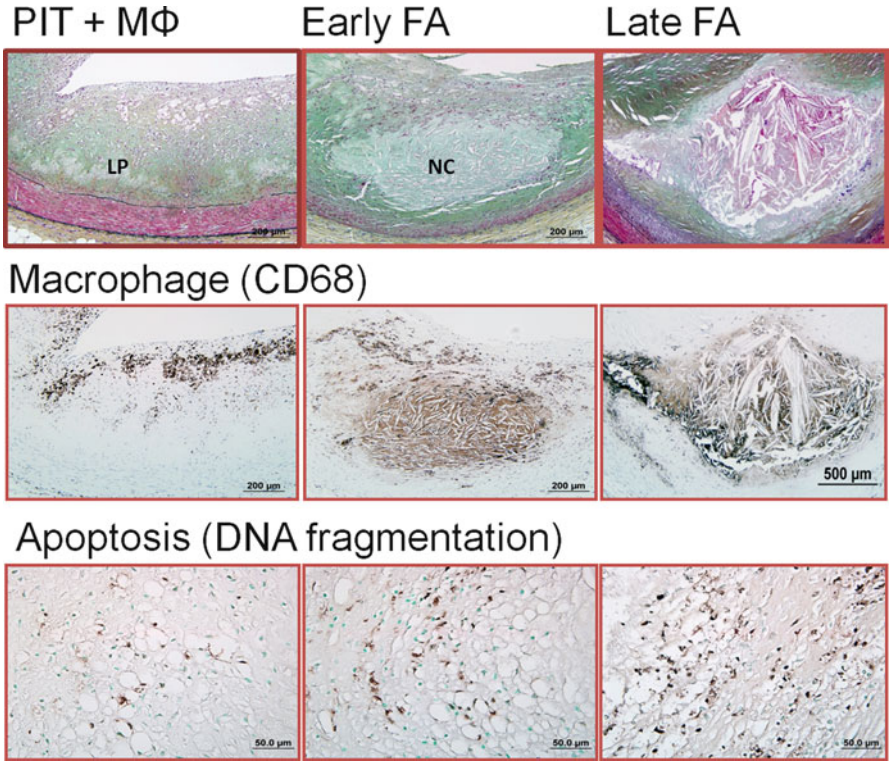

Fig. 2.3 Mechanism of necrotic core expansion in human coronary plaques. (*Left column*) Pathologic intimal thickening (PIT) is characterized by an underlying lipid pool (LP), which is devoid of macrophages (MΦ). The majority of macrophages when present are confined to the more luminal aspect of the plaque. Apoptotic cells are rarely seen in PIT. (*Middle column*) The presence of necrotic core (NC) infiltrated by CD68-positive macrophages characterizes the early stage of fibroatheroma (FA). Macrophages in early FA are capable of engulfing apoptotic bodies. (*Right column*) Late FA is represented by increased macrophage death and cell lysis. Free apoptotic bodies are commonly seen, possibly indicating defective clearance (efferocytosis) by resident macrophages

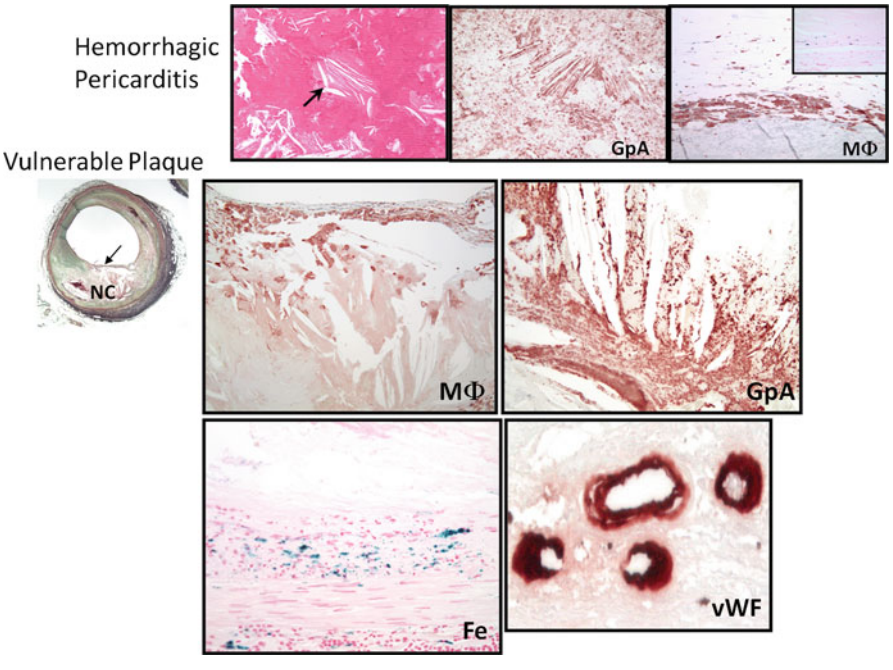

Fig. 2.4 Intraplaque hemorrhage in fibroatheroma with a late-stage necrotic core. Intraplaque hemorrhage leads to the accumulation of cholesterol monohydrate and increased lesion vulnerability. Illustrated in the *top panel* is the initial observation of hemorrhage in nonvascular sites showing accumulated free cholesterol. Note free cholesterol clefts in hemorrhagic pericarditis (*arrow*). Stained by Glycophorin A (GpA) which is specific for red blood cells, it is noted that positive staining surrounds the crystal structure of cholesterol. At the periphery of the hemorrhage, foamy macrophage (MΦ) were observed. The *bottom panel* shows a vulnerable plaque with macrophage positivity within the thin fibrous cap. GpA is strongly positive along with iron (Fe). Moreover, leaky microvessels within the plaque are detected as illustrated by the diffuse staining of von Willebrand factor (vWF) (reproduced with permission from Nakano et al. Vulnerable plaque. In: Zeev Vlodaver (ed) Coronary heart disease: clinical, pathological, imaging and molecular profiles. Springer)

other debris, influences both the biochemical composition and size of the necrotic core [23, 24].

The origin of intraplaque hemorrhage is also debatable between those claiming blood influx from luminal subsequent to plaque fissuring and proponents of leakage from intraplaque microcapillaries. However, we are in favor of the latter since often intraplaque erythrocyte extravasation is seen in the absence of plaque fissure and is associated with a high density of small vessels within the plaque. Further, some reports demonstrated the presence of incomplete mural cells coverage and dysfunctional endothelial cells of capillaries and arterioles with focal absence of basement membranes and poorly formed endothelial junctions [25]. It is possible that these immature or leaky vessels also allow diffusion of plasma proteins and diapedesis of leukocytes and erythrocytes spillage [26], which may serve as a driving force of further centripetal angiogenesis from the adventitia [27].

2.5 Hemoglobin Toxicity and Oxidative Stress

Intraplaque hemorrhage could potentially also recruit inflammatory cells [28]. The precise signaling pathways for the cellular response are not fully understood but it is postulated that hemoglobin–haptoglobin receptor CD163 on macrophages may be involved in the clearance of the complex with release of anti-inflammatory cytokines that may contribute to a decrease in inflammation [29]. However more importantly, our recent studies have elucidated the significance of oxidative stress associated with extravasated erythrocytes providing a rapid increase of hemoglobin (Hb) and continued inflammation in the intraplaque area [30]. Free Hb binds to and inactivates nitric oxide (NO), a potent molecule that plays a critical role in the regulation of smooth muscle vaso-reactivity and endothelial adhesion molecule expression, events that lead to inflammation within the vessel wall [31].

The function of haptoglobin (Hp) is primarily to handle hemoglobin released from red blood cells following intravascular or extravascular hemolysis. There are two common alleles at the Hp genetic locus denoted as 1 and 2, with two homozygous (1-1 and 2-2) and one heterozygous (2-1) genotype possible. There are functional differences between the Hp 1 and Hp 2 protein products in protecting against hemoglobin-driven oxidative stress with important functional and clinical significance. It is reported that there is a three- to fivefold increased risk of cardiovascular disease (CVD) in individuals with diabetes mellitus (DM) having the Hp 2-2 genotype as compared to DM individuals without the Hp 2-2 genotype. In particular, individuals with the Hp 2-2 genotype and diabetes mellitus appear to be at significantly higher risk of microvascular and macrovascular complications.

In atherosclerotic plaques, the primary route for clearance of the Hb–Hp complex involves the CD163 receptor expressed on immunosuppressive macrophages with M2 phenotype [32]. Physiologically low concentrations of hemoglobin (Heme) are cytoprotective as they induce the rapid upregulation of hemoxygenase-1 (HO-1). Excess pathological amounts of heme outstrip the ability of HO-1 to metabolize it so that residual heme (librating free iron) may act deleteriously on tissue by pro-oxidative and pro-inflammatory effects [33]. Also, when the capacity of protective hemoglobin-scavenging mechanisms has been saturated, levels of cell-free hemoglobin increase, resulting in the consumption of nitric oxide and resulting in clinical sequelae. NO plays a major role in vascular homeostasis and has been shown to be a critical regulator of basal and stress-mediated smooth muscle relaxation and vasomotor tone, endothelial adhesion molecule expression, and platelet activation and aggregation. Another product of excessive heme is bilirubin, which has potential antioxidant activity. Free ferrous iron has potential pro-oxidant activity, although this may be limited by its sequestration by ferritin.

2.6 Thin-Cap Fibroatheroma and Plaque Rupture

Thin-cap fibroatheroma (TCFA), traditionally designated as vulnerable plaque, is characterized by having a morphological appearance that resembles ruptured plaque [3]. TCFA generally contain a large necrotic core with overlying thin intact fibrous caps consisting mainly of type I collagen with varying degrees of macrophages and lymphocytes and very few, if any, α-actin-positive SMCs. The fibrous cap thickness is an indicator of plaque vulnerability and a TCFA is defined as having a cap thickness ≤ 65 μm since the thinnest portion of the remnant cap of a ruptured plaque was measured as 23 ± 19 μm, with 95 % of ruptured caps measuring ≤ 65μm [34]. As compared to ruptured plaque, TCFA tend to have smaller necrotic cores and less macrophage infiltration. Cross-sectional luminal narrowing is also typically less in TCFA compared to ruptures and occlusive thrombus generally shows greater underlying stenosis than lesions with non-occlusive thrombus [35].

It has been shown that the site of rupture usually occurs at its weakest point, often near shoulder regions. However, in our experience, this is not always the case as we have observed an equivalent number of ruptures at the mid portion of fibrous cap, especially in individuals who are dying during exertion [36]. Therefore, it is reasonable to speculate that several processes may be involved in the mechanisms of plaque rupture, e.g., fibrous cap degradation

by matrix metalloproteases (MMPs) [37], high shear stress [38], macrophage and smooth muscle cell death [39], and microcalcification and iron accumulation within the fibrous cap [40] all have been implicated.

Once plaque rupture occurs and necrotic contents are exposed to the flowing blood, this results in the triggering of the coagulation cascade in response to lipids, collagen, and tissue factors (TF). The luminal thrombus at the site of rupture is platelet rich (white thrombus) while proximal and distal to the rupture site, i.e., sites of propagation of the thrombus consist of a red thrombus composed of layers of fibrin and erythrocyte. A study of aspirated thrombi from patients presenting with AMI when examined by scanning electron microscopy (SEM) confirmed a decrease of platelet content and an increase of fibrin content as the duration of ischemia increased [41].

2.7 Plaque Erosion

Plaque rupture of an atherosclerotic plaque is the primary cause of AMI and SCD, occurring in 60–75 % of cases [42]. In the mid-1990s, our laboratory and that of van der Wal et al. reported an alternative mechanism of coronary thrombosis, referred to as "plaque erosion". In plaque erosion, the thrombus is confined to the luminal plaque surface with an absence of fissures or communication with the underlying necrotic core (when present), a finding validated by serial sectioning. In a study of 20 AMI patients, van der Wal et al. showed that the incidence of plaque ruptures (60 %) was more frequent than "superficial erosion" (40 %) [43]. In our series of 50 consecutive cases of sudden death due to coronary artery thrombosis plaque rupture was identified in 28 (56 %) cases, while superficial erosion was observed in 22 (44 %) cases, all of which had a smooth muscle cell and proteoglycan-rich underlying plaque [44]. In more recent studies, of AMI and SCD cases plaque erosion is identified as an important substrate of coronary thrombosis with its frequency being higher in women than men [45].

The term "erosion" was used since the luminal surface underneath the thrombus was devoid of endothelium. In addition, there were clear morphologic differences between rupture and erosion with plaque erosions having fewer macrophages and T-lymphocytes as compared to plaque rupture [44, 46]. In addition, eroded plaques tend to be more frequently eccentric with lesions rich in proteoglycan versican, hyaluronan, and type III collagen, unlike ruptured or stable plaques. Further, thrombi from erosion express a greater number of myeloperoxidase (MPO)-positive cells and have a higher incidence of distal micro-emboli than ruptures [47, 48]. Taken together, the above facts suggest a great necessity to better understand mechanistic differences between erosion and rupture, where different strategies may be required for the diagnosis and treatment of erosions.

2.8 Calcified Nodule

Calcified nodules is the least frequent cause of coronary thrombosis. It is characterized as a lesion with underlying calcification that is fragmented into small amorphous nodules on the luminal surface with surrounding fibrin, while the deeper portions more often show sheets of calcification. It morphologically resembles eruptive nodules (often multiple nodules with or without bone formation) protruding into the lumen, accompanied by a platelet-rich thrombus, which is usually non-occlusive. Little is known about the origin of nodular calcification. Histologically, fibrin is often present between the bony or calcified spicules, along with osteoblasts, osteoclasts, and inflammatory cells, indicating possible entrapment of circulating stem cells or cell transformation occurring from existing plaque cells [3]. Lesions with nodular calcification are more common in older individuals, more likely seen in males and chronic renal failure patients, and are preferentially found in tortuous middle right coronary or left anterior descending coronary arteries. They also appear to be more prevalent in the carotid arteries than coronary, which may be related to a greater frequency of calcification in carotid disease. The necrotic core is usually small, if present, in comparison to other atherothrombotic lesions.

2.9 Healed Plaque Rupture

The prevalence of silent plaque rupture or erosion in the clinical setting remains unknown as there are few studies that have demonstrated plaque progression following clinical events. Overall, it has been demonstrated in the NHLBI Dynamic Registry involving consecutive patients undergoing percutaneous coronary interventions (PCI) having a 6 % rate of nontarget lesion PCI by 1 year, and that the greater the coronary artery disease burden the higher the risk. Another recent study in patients who presented with an ACS and underwent PCI, major cardiovascular events in nontarget lesions were 11.6 % at 30 months. These lesions had angiographically mild disease, most often were TCFA, and were characterized by a large plaque burden and a small lumen area or some combination of these characteristics. Also, autopsy studies provide evidences that plaque progression defined as cross-sectional luminal narrowing occurs following repeated thrombotic events. Ruptured lesions with healed repair sites, referred to as the HPR, are discernible by breaks in the underlying old fibrous cap with a newly formed overlying tissue consisting of SMC surrounded by

proteoglycans and/or a collagen-rich matrix depending on the phase of healing [49]. It is likewise the case for healed erosion, i.e., similarly to that of rupture sites, erosion lesions heal and lead to luminal narrowing. Early stage of healing towards the lumen is characterized by lesions that are rich in proteoglycans and type III collagen, with an underlying necrotic core and ruptured fibrous cap rich in type I collagen.

Davies showed that the mechanism of plaque progression was through HPRs. The frequency of HPR correlates with the degree of luminal narrowing such that HPR was identified in 8 % of lesions with <20 % diameter stenosis, 19 % with 21–50 % diameter stenosis, and 73 % with >50 % stenosis [49]. We showed in patients with SCD that the incidence of HPR was 61 %; the percent luminal narrowing increased with increased number of healed rupture sites of previous ruptures. These data provided evidence that silent plaque ruptures is a form of wound healing that results in increased percent stenosis [50].

2.10 Calcified Stable Plaque (Fibrocalcific Plaque)

Although extent of calcification has been shown to be a predictor of diffuse coronary disease by CT, calcification of atherosclerotic plaque in SCD patients is observed in 80 % of patients, and the degree of calcification significantly varies from patient to patient and does not necessarily correlate with the disease severity or plaque vulnerability. Calcification is likely a consequence of multiple risk factors which include age/gender [51], renal function, diabetes [52], vitamin D levels and other aspects of bone metabolism [53], and genetic markers [54].

The initiation of calcification at least in man in atherosclerotic plaque is a marker of cell death, which is an essential component of all atherosclerotic plaques. The apoptotic smooth muscle cell is considered to be the earliest source of plaque calcification by an active or passive process involving calcification of the cell organelles referred to as matrix vesicles, which are observed as microcalcifications by histology, and only appreciated following utilization of special stains like von Kossa [55]. Macrophage cell death is thought to be another source of early calcium deposition. The calcified macrophages are present as small blocky calcifications and are morphologically distinct from those of SMCs. We have observed that the microcalcifications, derived from the apoptotic SMCs and macrophages, generally begin within the lipid pool and in "early" necrotic cores close to the luminal surface. It remains to be elucidated how the calcifications extend and lead to diffuse calcification involving other extracellular matrix proteins such as collagen and proteoglycans or through the expression of bone-forming proteins. The eventual transformation into plates of calcification that may appear as pipestem calcification, which involves necrotic core, collagen, and inflammatory cells, and in late stages even bone formation may be observed. Immunohistochemical studies and gene expression studies have demonstrated the presence of bone morphogenic protein, osteopontin, bone sialoprotein, and the osteoblast specific transcription factor for bone formation is highly expressed in the calcified arteries as compared to the control. In heavily calcified lesions which are regarded as burnt-out lesions, there is little if any macrophage infiltration and absence of other inflammatory cells. Nevertheless, a fair proportion of the calcification is passive, being purely degenerative without biological regulation and consists of calcium phosphate crystals [56].

3 Plaque Morphologies and Clinical Significance

In histological studies of patients dying of SCD, fresh thrombus has been reported in 50–75 % of cases while the remaining cases succumb to stable plaque with severe stenosis (>75 % cross-sectional luminal narrowing) of major coronary arteries. Of the various cases of fresh thrombus, the underlying pathologic lesions are mainly plaque rupture (60–75 %), followed by erosion (30–40 %), and calcified nodules (2–7 %) [3, 57–60]. In cases where death is attributed to plaque rupture, 70 % of cases show the presence of TCFAs at the site remote from the ruptured lesion. On the contrary, the incidence of TCFAs is markedly less (30 % of cases) where death is associated with stable plaques with severe stenosis. Also, the incidence of thin-cap fibroatheroma is highest in patient dying of plaque rupture, less in stable plaques, and least in patients dying with plaque erosion. The majority of TCFAs occur predominantly in the proximal portion of the three major coronary arteries; the proximal portion of the left anterior descending artery is the most frequent location (43 %) followed by the proximal right coronary artery (20 %) and the least frequent is the left circumflex artery (18 %) [35].

While plaque rupture may lead to unstable angina, myocardial infarction, or sudden death, however, rupture may also occur without causing symptoms. Silent ruptures heal and their repeated occurrence at the same location leads to greater luminal area stenosis with each new rupture. These lesions exhibit multiple necrotic cores separated by layers of collagen. These repeated thrombotic events contribute to gradual luminal narrowing and plaque progression. This significant increase in plaque burden and luminal narrowing due to previous repeated thrombosis often occurring silently in the absence of cardiac symptoms. The prevalence of silent episodes of rupture in living patients is unknown. In our experience, 61 % of SCD victims show at least one HRP lesion, where the incidence is greatest in the deaths from stable plaques with severe stenosis (80 %), followed by acute plaque rupture (75 %), and the least in plaque erosions (9 %) [50].

Although lipids along with other traditional risk factors play an essential role in the causation of coronary artery disease, the mechanistic link between lipid and disease remains unknown. Similarly, plaque progression studies in humans have been derived mostly from autopsy studies and for the most part clinical studies in the last century concentrated on study of luminograms and not the arterial wall where most of the disease of atherosclerosis is located. Our animal models have also failed to show events such as plaque rupture that occur commonly in man. There is no doubt we have learned many disease mechanisms from genetically altered mice but the atherosclerotic lesions in no way resemble those of man. Similarly, the animal models have not helped predict the responsiveness of newer treatments in humans. Therefore, identification of various plaque characteristics in man by invasive or noninvasive means may be the only way we are likely to enhance our knowledge of plaque types and plaque progression in man. We must continue to improve our imaging tools for the detection and characterization of coronary artery disease, targeting detailed plaque morphology or specific metabolic processes, e.g., local inflammation or biomarkers, which may permit strict monitoring of the activity of atherosclerotic disease. The development of noninvasive imaging device for screening purposes in asymptomatic individuals may prove an indispensable tool for the prediction and management of patients at high risk of clinical events.

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