

Review

Pathophysiology of Atherosclerosis Plaque Progression



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Atherosclerotic plaque rupture with luminal thrombosis is the most common mechanism responsible for the majority of acute coronary syndromes and sudden coronary death. The precursor lesion of plaque rupture is thought to be a thin cap fibroatheroma (TCFA) or “vulnerable plaque”. TCFA is characterised by a necrotic core with an overlying thin fibrous cap ($\leq 65 \mu\text{m}$) that is infiltrated by macrophages and T-lymphocytes. Intraplaque haemorrhage is a major contributor to the enlargement of the necrotic core. Haemorrhage is thought to occur from leaky vasa vasorum that invades the intima from the adventitia as the intima enlarges. The early atherosclerotic plaque progression from pathologic intimal thickening (PIT) to a fibroatheroma is thought to be the result of macrophage infiltration. PIT is characterised by the presence of lipid pools which consist of proteoglycan with lipid insudation. The conversion of the lipid pool to a necrotic core is poorly understood but is thought to occur as a result of macrophage infiltration which releases matrix metalloproteinase (MMPs) along with macrophage apoptosis that leads to the formation of a acellular necrotic core. The fibroatheroma has a thick fibrous cap that begins to thin over time through macrophage MMP release and apoptotic death of smooth muscle cells converting the fibroatheroma into a TCFA. Other causes of thrombosis include plaque erosion which is less frequent than plaque rupture but is a common cause of thrombosis in young individuals especially women <50 years of age. The underlying lesion morphology in plaque erosion consists of PIT or a thick cap fibroatheroma. Calcified nodule is the least frequent cause of thrombosis, which occurs in older individuals with heavily calcified and tortuous arteries.

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Introduction

Despite continuous advances in medical interventional and surgical therapies for the treatment of atherosclerotic coronary disease the latter remains the principal killer in the western and the developing worlds [1]. A better understanding of atherosclerosis is therefore needed in order to prevent its devastating effects world wide. Through the appointment of a consortium of investigators in the field of atherosclerosis the American Heart Association (AHA) classification was first published in 1994/1995 which helped towards a better understanding. We have modified the AHA classification as it was based on the premise that plaque rupture was the only mechanism responsible for coronary thrombosis however, our laboratory and that of van der Wal described the existence of plaque erosion [2,3]. Atherosclerotic plaque rupture and thrombosis remains the main cause of the majority of acute coronary syndromes and sudden coronary

death. The precursor lesion of plaque rupture is a thin cap fibroatheroma or “vulnerable plaque”. Erosion and calcified nodule being two other mechanisms for the presence of luminal thrombosis. In this review, we will discuss the pathophysiology of atherosclerosis plaque progression with emphasis on plaque progression.

Acute Coronary Syndromes and Luminal Thrombosis

Patients with acute coronary syndromes present with unstable angina, acute myocardial infarction with or without ST elevation, and sudden coronary death. Most of the acute coronary syndromes are believed to result from luminal thrombosis, which from post-mortem studies have been described to arise from three distinct morphologic entities: rupture, erosion, and calcified nodules (Fig. 1) [2]. The underlying mechanism of sudden coronary death from thrombi is highest from plaque ruptures (55–65%), followed by erosions (30–35%), and least frequent from calcified nodules (2–7%) [2]. A recent review on the pathology of ACS shows that the world wide incidence of thrombosis from plaque rupture is higher than

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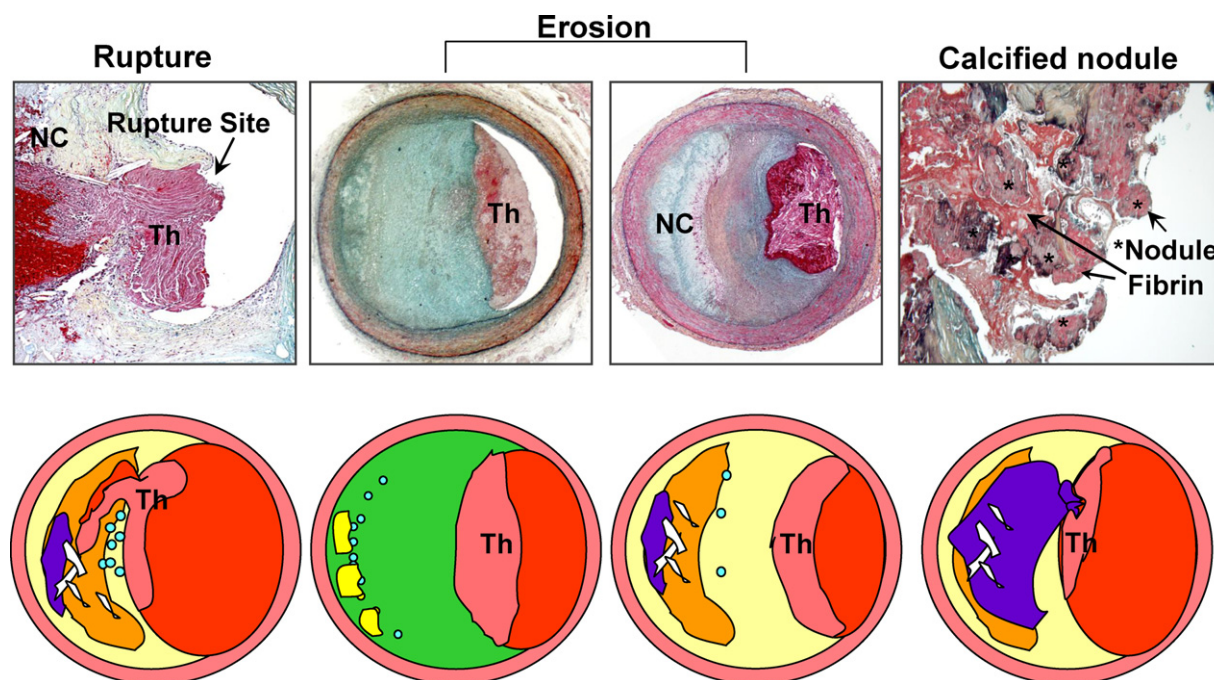


Figure 1. Atherosclerotic lesions with luminal thrombi. Coronary plaque features responsible for acute thrombosis comprise three different morphologies: rupture, erosion, and calcified nodules. Ruptured plaques are thin fibrous cap atheromas with luminal thrombi (Th). These lesions usually have an extensive necrotic core (NC) containing large numbers of cholesterol crystals and a thin fibrous cap ($<65\ \mu\text{m}$) infiltrated by foamy macrophages and T-lymphocytes. The fibrous cap is thinnest at the site of rupture and consists of a few collagen bundles and rare smooth muscle cells. The luminal thrombus is in communication with the lipid-rich necrotic core. Erosions occur over lesions rich in smooth muscle cells and proteoglycans. Luminal thrombi overly areas lacking surface endothelium. The deep intima of the eroded plaque often shows extracellular lipid pools, but necrotic cores are uncommon; when present, the necrotic core does not communicate with the luminal thrombus. Inflammatory infiltrate is usually absent, but if present, is sparse and consists of macrophages and lymphocytes. Calcified nodules are plaques with luminal thrombi showing calcific nodules protruding into the lumen through a disrupted thin fibrous cap. There is absence of an endothelium at the site of the thrombus, and inflammatory cells (macrophages and T-lymphocytes) are absent. Reproduced with permission from Virmani et al. [2].

73% however, this incidence is based on both hospital (AMI deaths) and coroner based sudden death autopsies of mostly older individuals [4]. In patients dying from acute myocardial infarction, the frequency of acute plaque rupture is higher (79%) than that of individuals dying sudden coronary death (65%). The frequency of plaque erosion in patients dying from AMI is lower (25%), but the incidence of erosion is higher in women than in men [2,5].

Plaque ruptures (Fig. 2) develop in a lesion with a large necrotic core (usually $>30\%$ plaque area) with an overlying thin disrupted fibrous cap, measuring $<65\ \mu\text{m}$ and is heavily infiltrated by macrophages and T-lymphocytes; a platelet rich luminal thrombus develops because of physical contact between flowing blood with highly thrombogenic necrotic core. By contrast, erosions are characterised by a luminal thrombus superimposed on a proteoglycan-rich matrix with mostly smooth muscle cells (SMCs) and very few inflammatory cells [5,6]. Calcified nodule, which is the least frequent cause of coronary thrombi, typically contains calcified plates along with calcified nodules that penetrate the lumen, with disruption of the overlying collagen layers and the endothelium; the thrombus is mostly non-occlusive [2].

The Classification of Atherosclerosis

Intimal Thickening and Fatty Streaks

The earliest vascular change described microscopically is intimal thickening (AHA Type I lesion), which consists of layers of smooth muscle cells and extracellular matrix. In autopsy specimens from 17 weeks of gestation to 23 months, it has been reported to occur in 35% of neonates where the intima/media ratio at birth is 0.1 and increases progressively to reach 0.3 by two years of age [7]. However, before the gestation of 30 weeks intima was rarely observed [7]. Smooth muscle cell proliferation was observed in the media before birth but was rare after birth, whereas the intima replication index was 2–5% [7]. Although intimal thickening is more frequent in atherosclerosis-prone arteries such as coronary, carotid, abdominal and descending aorta, and iliac artery [8], the change is considered adaptive (non-atherosclerotic) since the SMCs exhibit a very low proliferative activity later in life and show anti-apoptotic phenotype [9,10].

The termed “intimal xanthoma” or “fatty streak” (AHA Type II lesion), is a lesion primarily composed of abundant macrophage foam cells interspersed within a smooth muscle cells (SMC) and proteoglycan rich intima. Although

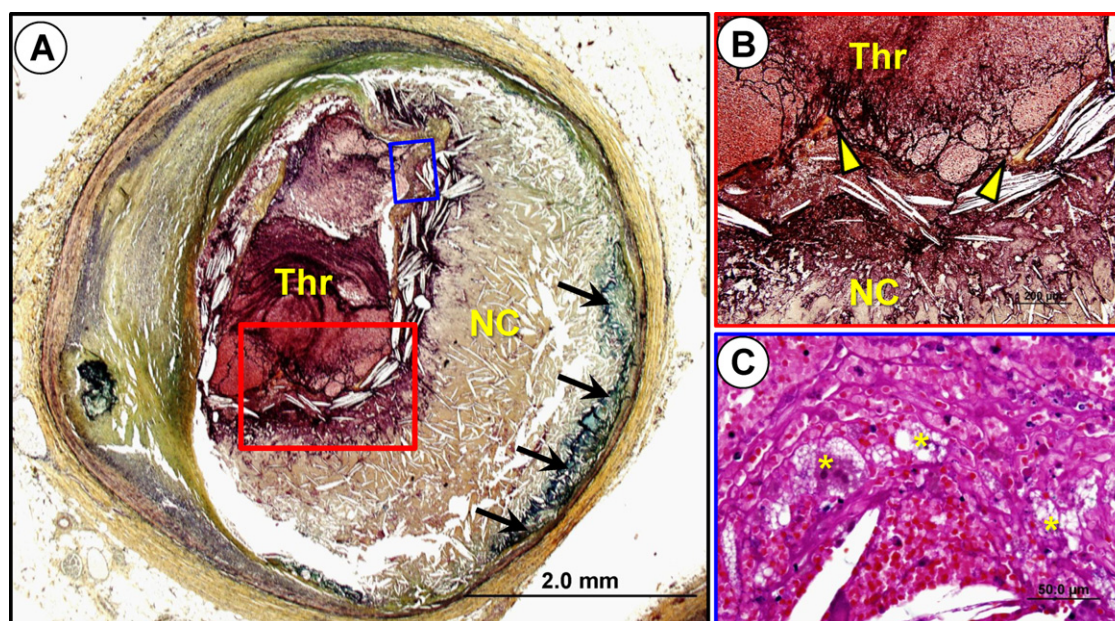


Figure 2. Plaque rupture. Cross-sectional photomicrograph of a coronary artery showing plaque rupture. (A) Note the presence of an acute occlusive luminal thrombus with an underlying large necrotic core (NC) and almost total absence of a fibrous cap. The medial wall is destroyed and near the base of the NC note the presence of calcification (arrows). (B) Higher-magnification image of the rupture site (red box in A). Thin fibrous cap is disrupted (arrowheads). (C) Higher-magnification of thrombus with cholesterol clefts (blue box in A), red cells, and foamy macrophages (asterisks).

Reproduced with permission from Falk et al. [4].

this entity is considered by AHA classification as the earliest lesion of atherosclerosis, our experience and reports of human and animal studies [11,12], show that this lesion is a reversible process (with little progressive tendencies). From the Pathobiologic Determinants of Atherosclerosis in Youth (PDAY) studied it is clear that lesions in the thoracic aorta, abdominal ventral aorta and mid right coronary lesions may regress with advancing age (15–34 years) [13]. Elegant studies of video microscopy have shown that foamy macrophages may leave the wall by passage between endothelial cells into the lumen [14].

Pathologic Intimal Thickening

The earliest of progressive lesions in our experience is the pathologic intimal thickening (PIT, AHA Type III lesion). This lesion is primarily composed of layers of smooth muscle cells in a proteoglycan-collagen matrix that is aggregated near the lumen with an underlying lipid pool consisting of an acellular area, rich in hyaluronan and proteoglycans (mainly versican) with lipid insudation [2]. There is an affinity for plasma lipoprotein, especially the low density lipoproteins to aggregate and attach to the proteoglycans of the extracellular matrix in the lipid pool [15,16]. Moreover, structural changes in the glycosaminoglycan chains on proteoglycans may represent an initial proatherogenic step that facilitates the binding and retention of atherogenic lipoproteins [17,18].

Another important hallmark of PIT is the variable accumulation of macrophages on the luminal aspect of the plaque (outside the lipid pool), although they are not observed in all lesions of PIT. We consider lesions

of PIT with macrophages as a more advanced stage of plaque development. Nakashima et al. described foamy macrophage accumulation in early lesions of plaque progression in coronary arteries near branch points [17]. While the precise nature of why macrophages accumulate in lesions of PIT is not fully understood, the expression of select proteins within lipid pools may play an important role. Furthermore, some lesions with PIT exhibit varying degrees of small free cholesterol clefts at the edge or within lipid pools. We assumed that the free cholesterol is derived from dead and dying smooth muscle cells however, this remains to be proven. Apoptotic SMCs within lipid pools are recognised by the presence of a thick basement membrane that surrounds the area previously occupied by the smooth muscle cell (cages of basal lamina). Also, observed within the lipid pool is the presence of microcalcification which are likely calcifying matrix vesicles [19,20].

Fibroatheroma

Fibroatheromas (AHA Type IV lesion) consist of an acellular necrotic core, which is distinguished from lipid pool areas of PIT as it is made up of cellular debris and is devoid of matrix which is best appreciated by sirius red staining. The necrotic core is covered by a thick fibrous cap consisting of smooth muscle cells in a proteoglycan-collagen matrix [2]. Our laboratory subclassifies fibroatheromas into those with “early” and “late” necrotic cores, because this distinction may enable us to better understand how necrotic cores evolve. Early necrotic core is identified by the presence of foamy macrophage seen infiltration into the luminal surface of the lipid pools which coincides

with the focal presence of calcifying macrophages and the presence of large free cholesterol clefts; there is definite lysis of the extracellular matrix. Lesions of early necrotic core characteristically exhibit proteoglycans, versican, and biglycan and hyaluronan, which are typically absent in late necrotic cores. Notably, the majority of macrophages within the areas of necrotic core display features consistent with apoptotic cell death although autophagic processes may also play a role [21].

The presence of free cholesterol is another discriminating feature of the late necrotic core and is partially attributed to apoptotic cell death of macrophages, in part regulated by ACAT1 [22]. The death of macrophages in the setting of defective phagocytic clearance of apoptotic cells is thought to contribute further to the development of plaque necrosis [22,23]. The late necrotic cores no longer stain for hyaluronan or proteoglycans and on sirius red stains there is absence of any collagenous matrix. The necrotic core area is surrounded by an overlying layer of thick “fibrous cap” composed mostly of type I and III collagen, proteoglycans and interspersed smooth muscle cells. The fibrous cap is critical for the maintenance of the integrity of the lesion and is subject to thinning, prior to rupture.

Plaque Rupture and Its Precursor Lesion – The Thin Cap Fibroatheroma (TCFA)

Thin-cap fibroatheroma (TCFA), traditionally designated as vulnerable plaque, is recognised by its morphologic characteristic that resembles a ruptured plaque. The main difference is not only the absence of a luminal thrombus and an intact thin fibrous cap (Fig. 3) [2,24], but a smaller necrotic core, and fewer macrophage infiltrating the thin fibrous caps. The thin fibrous cap is made up mostly of type I collagen and there is either an absence of, or only a few SMCs observed. The fibrous cap thickness is an indicator of plaque vulnerability which is defined to be $\leq 65 \mu\text{m}$ since the thinnest portion of the remnant ruptured cap was $23 \pm 19 \mu\text{m}$, with 95% of the TCFA caps measuring $\leq 65 \mu\text{m}$ [25]. OCT studies of ruptured caps have been measured in living patients and reported as $49 \mu\text{m}$ which is thicker than reported in autopsy studies [26], but this is not surprising as paraffin embedded sections are dehydrated and there is a 20–25% shrinkage of the tissue. TCFA differ from ruptured plaques on the basis of trends towards a smaller necrotic core, fewer macrophages within the fibrous cap and less calcification [27].

It is generally a prevailing thought that rupture of fibrous caps occurs at their weakest point, often near shoulder regions. However, we have observed an equivalent number of ruptures occurring at the mid portion of the fibrous cap. In individuals dying during exertion the rupture occurs mainly in the mid fibrous cap, whereas those dying at rest tend to rupture at shoulder regions [28]. Therefore, it is reasonable to speculate that different processes may be involved as catalysts for the rupture event. It has been shown that macrophages in the fibrous cap release matrix metalloproteinases (MMPs) [29] which further weaken the cap, along with high shear stress

[30], microcalcification and iron accumulation within the fibrous cap [31], and macrophage cell death [32], all contribute to the disruption of the fibrous cap. Once the fibrous cap ruptures, the necrotic core contents are exposed to the circulating blood, coagulation cascade involving platelets is activated in response to the exposure of lipids and tissue factors which are present in the necrotic core.

Biologic Markers of Plaque Progression

Today atherosclerosis is considered an inflammatory process that occurs as a response to the accumulating lipid within the arterial wall. It begins with elevation of plasma cholesterol levels which result in changes in the arterial endothelial cell permeability that allow the insudation of lipids, especially “cholesterol-containing low-density lipoproteins (LDL)” into the arterial wall where they bind to the extracellular proteoglycan rich matrix and aggregate (Fig. 4). Also, circulating monocytes now begin to adhere to the endothelial cells that express adhesion molecules, like vascular adhesion molecule-1 (VCAM-1) and selectins which result in the migration of monocytes via diapedesis between endothelial junctions and reside in the subendothelial space [33]. The monocytes then acquire characteristics of macrophages and convert to foamy macrophages. The prolonged residence of LDL particles in the subendothelial space results in their oxidation and other chemical modification. LDL oxidation is promoted in vitro by monocytes, endothelial cells, and smooth muscle cells. Oxidised LDL is a potent chemoattractant and induces the secretion of macrophage-chemotactic protein 1 (MCP-1) by endothelial cells [34,35]. Furthermore, macrophages express several scavenger receptors (SR) [SR A and B1, CD36, CD68 and scavenger receptor for phosphatidylserine, and oxidised LDL] which can bind a broad spectrum of ligands, including modified lipoproteins, native lipoproteins, and anionic phospholipids, many of which facilitate the massive accumulation of intracellular cholesterol [36].

While immune reactions involve various cell types, mononuclear cells play a critical role in atherosclerosis processes. Macrophages express various receptors [SR A and B1, CD 36, CD68, and Toll-like receptors (TLRs)] that recognise molecular patterns foreign to the body like bacterial pathogens. It has been shown that certain forms of bacterial toxins bind to SR-AI and SR-AII in vitro and are cleared from the circulation in vivo by SRs [37]. Toll-like receptors (TLRs) are one of those receptors that have been recognised to play an important role in many cardiovascular pathologies [38]. TLRs activate the MyD88/IRAK signalling cascade which activate the proinflammatory transcription factor nuclear factor kappa-B (NF- κ B) and the mitogen-activated protein kinase (MAPK) pathway resulting in the production of cytokines that augment local inflammation and smooth muscle cell proliferation [39,40]. Tumour necrosis factor- α (TNF- α) is released by the activation of TLR-2 and TLR-4 from circulating cells in patients with established atherosclerosis [41]. Also, TLR-4 is identified as the signalling receptor for endotoxins

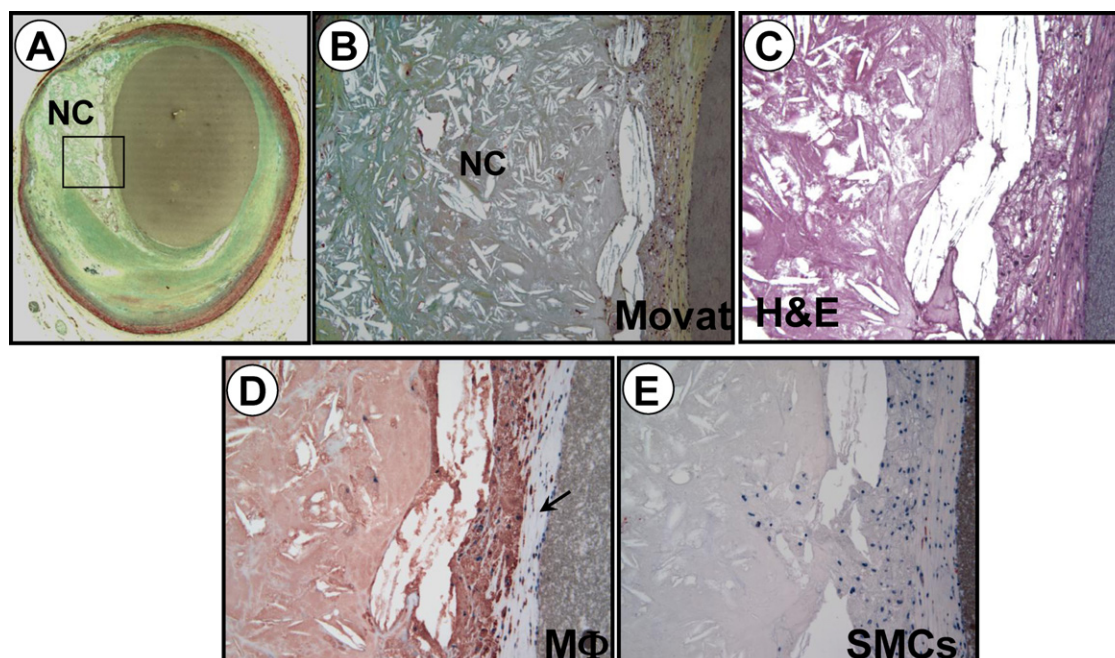


Figure 3. A non-haemodynamically limiting thin-cap fibroatheroma. (A) Low power view of a thin-cap fibroatheroma (TCFA) with a relatively large eccentric necrotic core (NC) and an overlying thin fibrous cap ($<65\ \mu\text{m}$); the greyish material in the lumen is barium gelatin (Movat pentachrome, $20\times$). (B) Image of the boxed area in panel A showing an advanced necrotic core with a loss of matrix containing numerous cholesterol clefts and cellular debris ($100\times$). (C) High power view of a fibrous cap heavily infiltrated by inflammatory cells (haematoxylin and eosin, $200\times$). (D) Numerous CD-68 positive macrophages are localised to the fibrous cap ($200\times$). (E) The fibrous cap shows rare α -actin positive smooth muscle cells (SMCs) ($200\times$).

Reproduced with permission from Kolodgie et al. [24].

and is expressed by macrophages in lipid-rich atherosclerotic plaques [42]. The uptake of oxidised LDL transforms macrophages into foam cells, which proliferate in the presence of MCP-1 and macrophage colony stimulating factor (MCSF) [33]. Macrophages produce many cytokines (interleukin-1, $\text{TNF-}\alpha$) that regulate the function of various cells involved in the process of atherosclerosis. Some cytokines may contribute to lymphocyte recruitment and these include CXC chemokines induced by interferon- γ [43]. The initial activation of T-cells requires a strong stimulus delivered by dendritic cells (a specialised macrophage), or via memory T-cells, which have a lower activation

threshold and require reduced amounts of antigen [44]. Endothelial cells are also critical in the inflammatory response that leads to leucocyte recruitment, increased permeability, oedema, and many other processes involved in atherosclerosis [36]. T-cell activation leads to the expression of CD40 (CD40L/CD154). CD40L (CD154) on the T cell binds CD40 on the macrophage cell surface [33,44–46], which results in increased macrophage expression of CD40 and TNF receptors that help increase the level of activation. While activation of CD40 on monocytes leads to the expression of LFA-1 and ICAM-1, ligation of CD40 on endothelial cells triggers the expression of leucocyte

Table 1. Morphometric Analysis of 365 Plaques in Coronary Arteries from Patients Who Died Suddenly of Coronary Causes.

Type of Plaque	No. of Plaque	Glycophorin A Score [†]	Iron Score [†]	Size of Necrotic Core mm^2	Extent of Macrophage Infiltration
Plaque with pathologic intimal thickening but with no necrotic core	129	0.09 ± 0.04	0.07 ± 0.05	–	0.002 ± 0.001
Fibroatheroma					
Core in early stage of necrosis	79	0.23 ± 0.07	0.17 ± 0.08	0.06 ± 0.02	0.018 ± 0.004
Core in late stage of necrosis	105	$0.94 \pm 0.11^{\ddagger}$	$0.41 \pm 0.09^{\ddagger}$	$0.84 \pm 0.08^{\ddagger}$	$0.059 \pm 0.007^{\ddagger}$
Thin-cap fibroatheroma	52	$1.60 \pm 0.20^{\ddagger}$	$1.24 \pm 0.24^{\ddagger}$	$1.95 \pm 0.30^{\ddagger}$	$0.142 \pm 0.016^{\ddagger}$

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[†] Scores can range from 0 to 4, with higher scores indicating greater proportions of the analyst.

[‡] $P < 0.001$ for the comparison with fibroatheromas whose cores were in an early stage of necrosis.

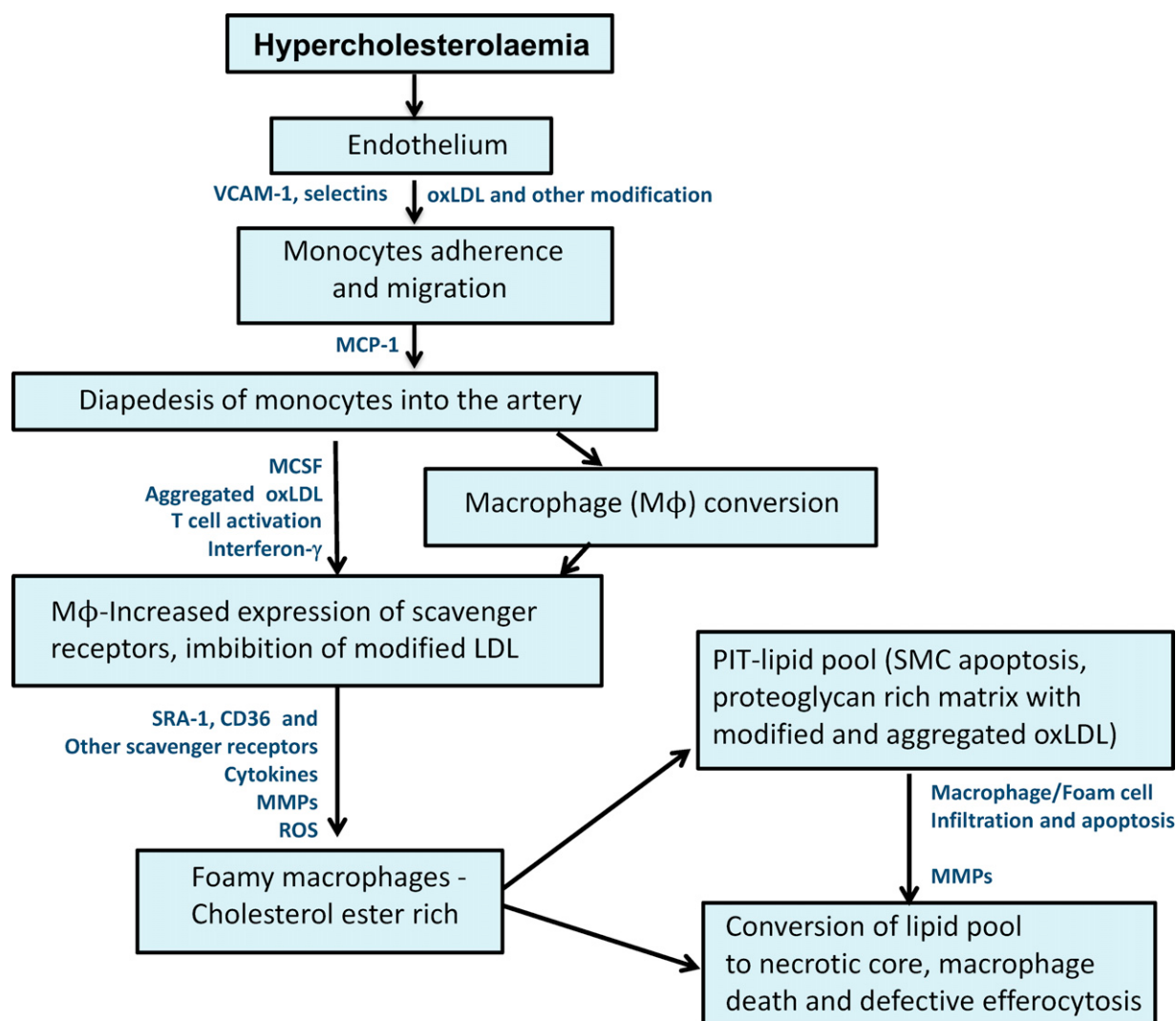


Figure 4. Sequence of events that lead to the atherosclerotic change and its progression. Hypercholesterolaemia is the trigger for the initiation of atherosclerosis. The monocyte rolling and attachment to endothelial is facilitated by expression of VCAM-1 (vascular cell-adhesion molecule-1), and selections. Oxidisation and other modifications of LDL induces the secretion of MCP-1 (macrophage-chemotactic protein-1). In the arterial intima, the monocytes undergo maturation into macrophages. Macrophages express scavenger receptors such as SRA (scavenger receptor A) and CD36, that facilitate the uptake of modified LDL and the conversion to foamy macrophages that are rich cholesterol esters and free fatty acids. Monocytes/macrophages proliferate in the presence of MCP-1 and macrophage colony stimulating factor (MCSF). After antigen-specific activation T-cell enter the intima secrete interferon- γ which send signals that help exaggerate the inflammatory response and sustain it. Macrophages excrete MMPs (matrix metalloproteinases), which are further enhanced by the presence of ROS (reactive oxygen species), which then facilitate the breakdown of collagen to allow migration of cells within the plaque. Foamy macrophages infiltrate pathological intimal thickening (PIT) lesion and the lipid pool areas that are formed by the SMC apoptosis, and proteoglycan accumulation. Heading and aggregation of oxLDL and macrophage infiltration induces the conversion of lipid pool to necrotic core, and with in the necrotic core macrophage death and defective efferocytosis both contributing to the sustained presence of CD 40 and CD40 ligand.

adhesion molecules such as VCAM-1, E-selectin, and ICAM-1 [46,47]. Genetically modified ApoE deficient mice that also lack CD40L or those treated with neutralising anti-CD40L antibody develop reduced levels of atherosclerosis on high-cholesterol diet [48]. Furthermore, several investigators have shown activation of endothelial cells and angiogenesis by CD40L and release of several matrix metalloproteinases (MMPs) and expression of vascular endothelial growth factor (VEGF), Cox-2, and fibroblast growth factors [44,46,47,49]. The loss of

fibrillar collagen by active MMPs is considered to be the main mechanism of the fibrous cap thinning. The various MMPs that are found in macrophage rich regions include MMPs-1, -2, -3, -8, -9, -11, -12, -13, -14, and -16 [50]. However, MMPs-1, -3, and -8, are found in areas of cleaved collagen, suggesting the fibrous cap thinning may occur through the expression of these MMPs [50]. Since CD40 and CD40L have been shown to co-localise with MMPs in human atheroma, CD40 and CD40L may also contribute to disruption of the fibrous cap [33,47].

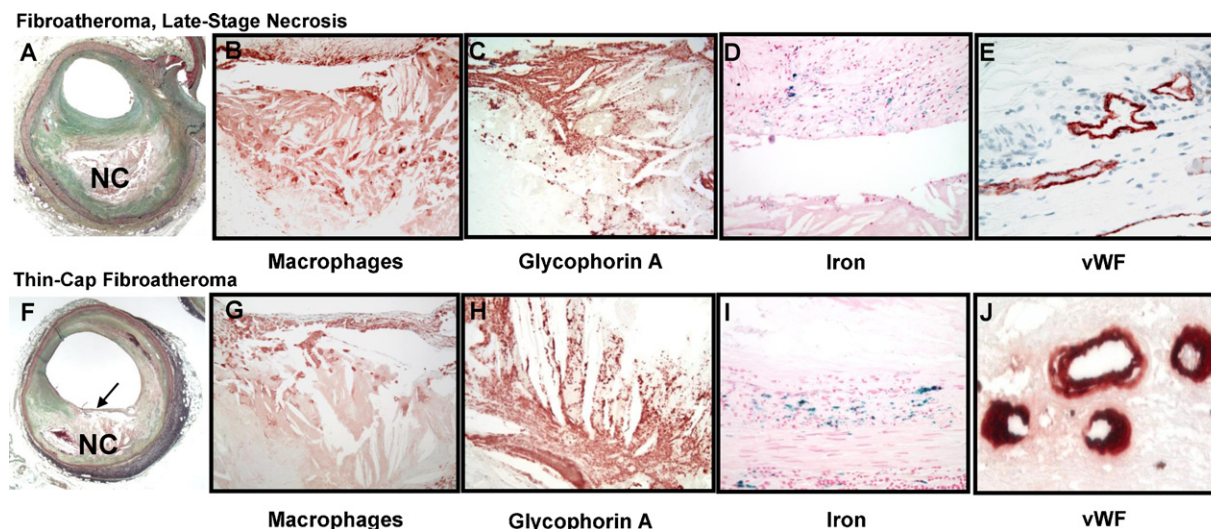


Figure 5. Intraplaque haemorrhage in fibroatheroma with a core in a late stage of necrosis (Panels A, B, C, D, and E) and thin-cap fibroatheroma (Panels F, G, H, I, and J). Panel A shows a low-power view of a fibroatheroma with a late-stage necrotic core (NC) (Movat pentachrome, 20×). Panel B shows intense staining of CD68-positive macrophages within the necrotic core (200×). Panel C shows extensive staining for glycophorin A in erythrocyte membranes localised with numerous cholesterol clefts within the necrotic core (200×). Panel D shows iron deposits (blue pigment) within foam cells (Mallory's stain, 200×). Panel E shows microvessels bordering the necrotic core with perivascular deposition of von Willebrand factor (vWF) (400×). Panel F shows a low-power view of a fibroatheroma with a thin fibrous cap (arrow) overlying a relatively large necrotic core (Movat pentachrome, 20×). The fibrous cap is devoid of smooth-muscle cells (not shown) and is heavily infiltrated by CD68-positive macrophages (Panel G, 200×). Panel H shows intense staining for glycophorin A in erythrocyte membranes within the necrotic core, together with cholesterol clefts (100×). Panel I shows an adjacent coronary segment with iron deposits (blue pigment) in a macrophage-rich region deep within the plaque (Mallory's stain, 200×). Panel J shows diffuse, perivascular deposits of von Willebrand factor in microvessels, indicating that leaky vessels border the necrotic core (400×).

Reproduced with permission from Kolodgie et al. [58].

Necrotic Core Enlargement is Critical for Plaque Rupture

It has been shown that macrophage infiltration is the first step towards the eventual formation of atherosclerotic plaque [2]. In vitro studies have shown that LDL uptake by macrophages is facilitated by a two-step oxidation process [51,52]. The first step begins with mild oxidation of lipid followed by apolipoprotein B oxidation, a modification required for its recognition by the scavenger receptor, which is unaffected by the cholesterol content of the cell [34]. Oxidised cholesterol is delivered to the lysosomes where the oxidised-LDL is hydrolysed to free cholesterol and free fatty acids, the excess cholesterol undergoes re-esterification by acyl coenzyme A:acylcholesterol transferase (SOAT; also known as ACAT) [36]. The excess free cholesterol produced by the macrophage is exported out of the cell most likely via the SR-B1 receptor or other mechanism to HDL particles [36,53]. Esterified cholesterol accumulates in the cytosol of the macrophage to form intracellular lipid droplets seen in foam cells [36]. As the plaque progresses, the free cholesterol content of lesion increases while cholesterol esters decrease [54]. In human aortic atherosclerotic plaques, the progression from a nondisrupted to a disrupted lesion was associated with an increase in free cholesterol, cholesterol esters, and the ratio of free-to-esterified cholesterol with no change in triglyceride content [54]. Furthermore, the percentage of cholesterol clefts is greater in lesions with ruptured

plaques as compared to those of erosion or stable plaques [2,27].

In the early to mid twentieth century, several leading pathologists (Wartmann 1938, Winternitz 1938, Patterson 1954) put forth the hypothesis that intraplaque haemorrhage is a major contributor to the progression of coronary atherosclerosis [55–57]. However, the mechanism of plaque haemorrhage was not elucidated. In an effort to further understand the influence of intraplaque haemorrhage on lesion progression, our laboratory examined lesions from PIT to fibroatheroma to thin cap fibroatheroma for the presence of haemorrhagic events [58]. A greater frequency of previous haemorrhages in coronary lesions prone to rupture (as detected by glycophorin A) relative to lesions with early necrotic cores or plaques with PIT was observed (Table 1) (Fig. 5) [58]. The degree of reactivity of glycophorin A and iron deposition in the plaque corresponded to the increasing size of the necrotic core, and macrophage density, suggesting that haemorrhage itself serves as an inflammatory stimulus [58]. The cause of intraplaque haemorrhage was considered to initiate from disruption of the thin-walled microvessels that are lined by a discontinuous leaky endothelium without the presence of supporting smooth muscle cells [59,60]. Although pathologic examination of unstable lesions shows that intraplaque haemorrhage and plaque rupture are associated with an increased density of microvessels [28,61,62], the precise mechanisms involved in red blood cells' leakage into the necrotic core are

poorly understood. We have shown diffuse perivascular staining of von Willebrand (vWF) factor of intraplaque vasa vasorum and the presence of erythrocyte membranes within necrotic cores, which suggest microvascular disruption or incompetent endothelium as a source of erythrocyte-derived cholesterol [58]. Furthermore, some reports demonstrated dysmorphogenesis of the vasa vasorum with absence of mural cells and poor endothelial cell junction [63]. These immature or leaky vessels therefore allow diffusion of plasma molecules and diapedesis of leucocytes as well as erythrocytes [64], which are the driving force of further centripetal angiogenesis from the adventitia [65].

Moreover, recent studies have revealed the significance of oxidative stress associated with extravasated red blood cells providing a rapid influx of haemoglobin and macrophage infiltration in the intraplaque area. Free haemoglobin can also bind and inactivate nitric oxide (NO), a potent signal molecule that plays a critical role in the relaxation of smooth muscle and reduced expression of endothelial adhesion molecules, eventually leading to cell injury [66]. A major protective mechanism against direct toxicity of haemoglobin is the presence of haptoglobin, an abundant serum protein whose major function is to bind excess haemoglobin. In atherosclerotic plaques, the primary route for clearance of the haemoglobin is through haemoglobin–haptoglobin complex which bind to the CD163 receptor expressed on macrophages with a subtype of M2 phenotype [67]. Another possible means of protection is the presence of heme oxygenase-1 which has anti-oxidative, anti-inflammatory, anti-apoptotic and possibly immune-modulatory properties and is expressed in endothelial cells, macrophages and SMCs [68].

Arterial Remodelling in Coronary Atherosclerosis

The seminal work by Glagov et al. in the late 1980s elegantly showed that the vessel size increases with increasing plaque burden, such that the lumen remain similar to normal adjacent artery segments. This compensatory enlargement can only occur as long as the plaque burden remains <40% [69]. The absence of loss of lumen is due to compensatory enlargement of the vessel [69,70]. Postmortem and intravascular ultrasound studies have revealed that positive remodelling of the coronary arterial wall is associated with an increasing lipid content and macrophage infiltration as well as unstable plaque features such as acute rupture, intraplaque haemorrhage, and TCFA [71,72]. Not only is there correlation of macrophage infiltration and compensatory enlargement but also the medial smooth muscle cells undergo apoptosis. Conversely, lesions of erosion, chronic total occlusion have been shown to exhibit negative remodelling as there are few inflammatory cells (Fig. 6) [71]. Thus, arterial remodelling may represent an important surrogate for the detection of lesion vulnerability. The expression of activated MMPs likely influences the extent of arterial remodelling and the genetic manipulation of individual MMPs has led to the evidence that MMP-9 may be the dominant MMP in vascular remodelling along with

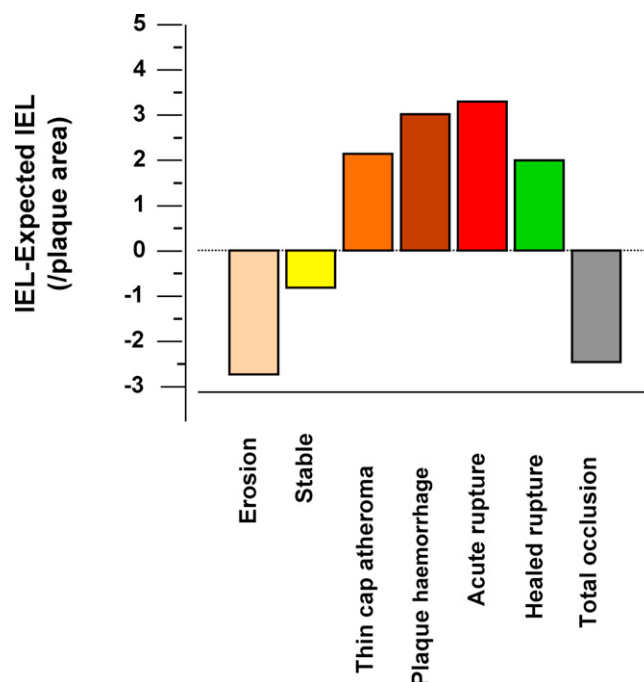


Figure 6. Remodelling by type of plaque. The IEL increased adjusted for plaque area is plotted by plaque type. Plaque haemorrhage, including ruptures, showed significantly greater remodelling than fibrous plaques ($P=0.003$, means table with Fisher's post hoc test) and total occlusions ($P=0.03$). Fibrous plaques showed less remodelling than thin-cap atheromas and healed ruptures ($P<0.01$). Reproduced with permission from Burke et al. [71].

MMPs-1, -2, -3, -8, and -14 [73]. However, a recent study suggests that MMP-3 and MMP-9 may be beneficial with regard to plaque stabilisation by promoting fibrous cap formation [74]. Further studies are needed to elucidate the role of MMPs in human coronary arteries.

Plaque Erosion

Plaque erosion is defined as an acute thrombus that is in direct contact with the intima, which is rich in SMCs and proteoglycan matrix with an absence of endothelial lining. The underlying plaque in erosions consists of either PIT or fibroatheroma with a thick fibrous cap, both occurring with equal frequency. We speculate that coronary vasospasm may be involved in its pathophysiology since macrophages and lymphocytes are typically absent, also the media is noted to be intact while in ruptures the media is often destroyed, especially the internal elastic membrane. The plaque in close proximity to the thrombus in erosion lesions is rich in versican, hyaluronan and type III collagen unlike rupture or stable plaque which are rich in type I collagen and biglycan and decorin [6,75]. The erosion lesions are most often eccentric and infrequently calcified. It is hypothesised that a selective accumulation of hyaluronan in eroded plaques may promote deendothelialisation and platelet aggregation. It has been shown that hyaluronan can directly promote the polymerisation of fibrin, which may facilitate SMC migration and plaque

progression. Our laboratory has shown that the eroded site shows minimal inflammation characterised by few or absent macrophages and lymphocytes [2,6], whereas van der Wal et al. reported inflammation at the site of erosion [3].

Plaque erosion accounts for 25–35% of coronary thrombi in patients dying from acute myocardial infarction or sudden coronary death [2,25]. The risk factors for erosion are considered to be different from those of rupture [25]. In our first publication which involved men dying from sudden coronary death, ruptured plaque had higher plasma total cholesterol (TC), lower high-density lipoprotein cholesterol (HDL-C), and higher TC/HDL-C ratio as compared to plaque erosion [25], while only TC correlated with plaque rupture in women, which was shown in a separate study [76]. Consistently smoking is associated with acute thrombosis, and that smoking is a significant risk factor for plaque erosion in women with coronary disease who died suddenly [76]. From our sudden coronary death registry it has been observed that eroded plaques have greater proportion of female, of younger age, less % stenosis, less calcification, with less plaque burden and thrombus as compared to ruptured plaques [6]. Plaque

erosion accounts for over 80% of thrombi occurring in women <50 years of age, which is reversed beyond 50 years.

In sudden coronary death victims, intramyocardial microemboli were more frequently observed in erosions (71%) than in plaque rupture (42%) [77]. Moreover, in a separate study we showed that greater than 85% of coronary thrombi in erosions exhibited late stages of healing, whereas in ruptures only one-half of thrombi showed any healing [78]. In an autopsy study coronary thrombi in eroded plaques have been shown to contain a higher density of myeloperoxidase-positive cells than those of ruptured plaques [79]. Moreover, in living patients the circulating blood myeloperoxidase levels are elevated in patients with acute coronary syndromes with erosion as compared to those with rupture, suggesting that it may be possible to separate rupture from erosion by inflammatory biomarkers and presence of plaque erosion can be further confirmed by OCT. Nevertheless, large systematic studies involving many patients are needed to confirm mechanistic differences between erosion and rupture, and perhaps different strategies may be indicated for the treatment of these erosive lesions.

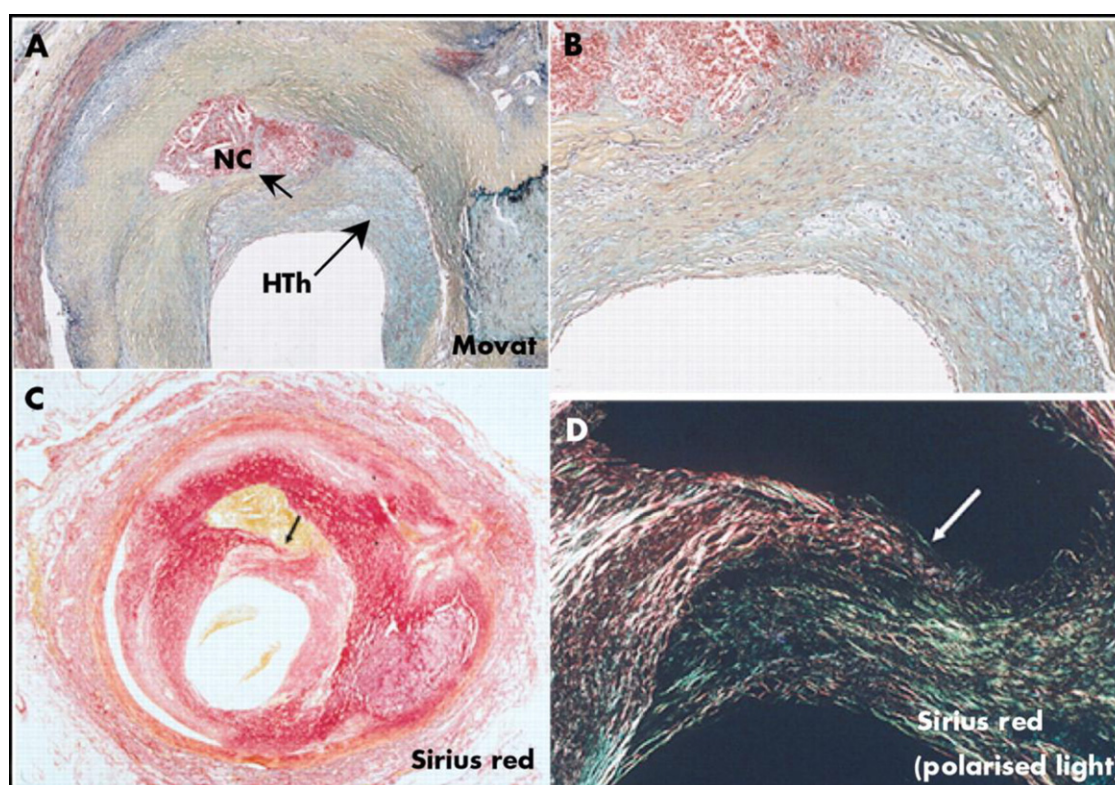


Figure 7. Healed plaque rupture. (A) Areas of intraintimal lipid-rich core with haemorrhage and cholesterol clefts; an old area of necrosis (NC) is seen underlying a healed thrombus (HTH). (B) Higher magnification showing extensive smooth muscle cells within a collagenous proteoglycan-rich neointima (healed thrombus) with clear demarcation from the fibrous region of old plaque to right. (C and D) Layers of collagen by Sirius red staining. (C) Note area of dense, dark red collagen surrounding lipid haemorrhagic cores seen in corresponding view in A. (D) Image taken with polarised light. Dense collagen (type I) that forms fibrous cap is lighter reddish yellow and is disrupted (arrow), with newer greenish type III collagen on right and above rupture site. (A and B) Movat pentachrome. Reproduced with permission from Burke et al. [82].

Calcified Nodule

Calcified nodule, the least frequent cause of coronary thrombi, is a lesion that occurs in highly calcified arteries. It consists of areas of fragmented calcified sheets that form small calcified nodules that are surrounded by fibrin and have a small luminal thrombus. The eruptive calcified nodules are usually eccentric, protruding into the lumen, and there is an absence of endothelium and collagen above the nodules of calcium with a platelet-rich thrombus which is usually non-occlusive. Although the mechanisms of nodular calcification remain unknown, by histology, fibrin is often present between the calcified spicules, along with rare osteoclasts and inflammatory cells, indicating that at site of calcified sheet cracking and breaks in the calcified region with intraplaque haemorrhage and cell transformation may be involved [2]. Lesions with nodular calcification are more common highly calcified tortuous arteries, in older individuals, in males, and are preferentially found in the middle right coronary or the left anterior descending coronary arteries. We believe that these lesions occur more frequently in the carotid arteries than the coronary and might be related to plaque haemorrhage and calcification.

A recent report from the PROSPECT (Providing Regional Observations to Study Predictors of Events in the Coronary Tree) study demonstrated that patients with “IVUS-detected calcified nodule” had fewer major adverse cardiac events than those without calcified nodule [80]. However, the PROSPECT study did not distinguish “calcified nodule” from “nodular calcification”. The previously reported calcified nodules in the pathologic studies require the presence of luminal thrombus [2]. Also VH-IVUS is unable to detect the presence of thrombus. “Non-thrombotic calcified nodules” should be discriminated from “calcified nodules”, and non-thrombotic calcified nodules have been described by us as “nodular calcification”. The results of the PROSPECT study suggest that lesions with nodular calcification are frequent and are not at high-risk of future events.

Healed Plaque Ruptures

Morphologic studies suggest that plaque progression beyond 40–50% luminal narrowing occurs secondary to repeated plaque ruptures, which most often are clinically silent when they occur in less severely narrowed arteries. Ruptured lesions with healed repair sites are called healed plaque rupture (HPR) (Fig. 7). HPR are typically detected by the identification of breaks in the fibrous cap with an overlying repair reaction consisting of a proteoglycan-rich mass with type III collagen in the early phase but type I collagen at late time points after the event of rupture (>1 year). Davies et al. showed that the frequency of HPRs increases as the lumen narrows [81]. We also showed that 61% of hearts from sudden coronary death victims show HPRs [82]. The incidence of HPRs is highest in stable plaques (80%), followed by acute plaque rupture (75%), and least frequent in erosions (9%).

Conclusions

The primary cause of luminal thrombosis is predominately from plaque rupture, followed by plaque erosion, and least frequent from calcified nodule. The precursor lesions with the potential to rupture are called ‘vulnerable’ plaques or “thin cap fibroatheroma” which may not be severely narrowed and could represent an appropriate risk-assessment for patients at risk for future coronary events. Macrophages along with T-cells play a pivotal role in the progression of atherosclerotic plaque. Intraplaque haemorrhage is a major contributor to the enlargement of the necrotic core. There are no clinical trials confirming that successful treatment of a vulnerable plaque results in the reduction of future cardiovascular events. Additional refinements to currently available detection modalities for the identification of vulnerable lesions and newer therapies or strategies with careful monitoring of all aspects of disease anatomy and epidemiology will be needed. The pathophysiology of atherosclerotic plaque progression as presented in this review are meant to encourage better understanding by clinicians such that targeting plaque vulnerability and risk reduction can be accomplished in ACS patients.

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