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REVIEW

Basic science for the clinician

Update on acute coronary syndromes: the pathologists' view

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Although mortality rates from coronary heart disease in the western countries have declined in the last few decades, morbidity caused by this disease is increasing and a substantial number of patients still suffer acute coronary syndrome (ACS) and sudden cardiac death. Acute coronary syndrome occurs as a result of myocardial ischaemia and its manifestations include acute myocardial infarction and unstable angina. Culprit plaque morphology in these patients varies from thrombosis with or without coronary occlusion to sudden narrowing of the lumen from intraplaque haemorrhage. The coronary artery plaque morphologies primarily responsible for thrombosis are plaque rupture, and plaque erosion, with plaque rupture being the most common cause of acute myocardial infarction, especially in men. Autopsy data demonstrate that women <50 years of age more frequently have erosion, whereas in older women, the frequency of rupture increases with each decade. Ruptured plaques are associated with positive (expansive) remodelling and characterized by a large necrotic core and a thin fibrous cap that is disrupted and infiltrated by foamy macrophages. Plaque erosion lesions are often negatively remodelled with the plaque itself being rich in smooth muscle cells and proteoglycans with minimal to absence of inflammation. Plaque haemorrhage may expand the plaque rapidly, leading to the development of unstable angina. Plaque haemorrhage may occur from plaque rupture (fissure) or from neovascularization (angiogenesis). Atherosclerosis is now recognized as an inflammatory disease with macrophages and T-lymphocytes playing a dominant role. Recently at least two subtypes of macrophages have been identified. M1 is a pro-inflammatory macrophage while M2 seems to play a role in dampening inflammation and promoting tissue repair. A third type of macrophage, termed by us as haemoglobin associated macrophage or M(Hb) which is observed at site of haemorrhage also can be demonstrated in human atherosclerosis. In order to further our understanding of the specific biological events which trigger plaque instability and as well as to monitor the effects of novel anti-atherosclerotic therapies newer imaging modalities *in vivo* are needed.

Keywords Acute coronary syndrome • Plaque rupture • Plaque erosion • Calcified nodule • Vulnerable plaque

Introduction

For the past decades, the mortality of coronary heart disease (CHD) has declined substantially in many affluent countries.[1] Previously, myocardial infarction (MI) was a disease with a dire short-term prognosis, but now the survival after MI has improved substantially, with the consequence of increasing CHD prevalence, chronic disability and treatment costs.[1–3] Coronary heart disease is projected to remain a leading cause of death and disability not only in affluent countries but globally for many years to come.[3] Effective prevention strategies are needed if we are to limit the growing burden of CHD.

Coronary heart disease is nearly always caused by coronary atherosclerosis with or without luminal thrombosis and vasospasm.[4,5] Atherosclerosis alone may cause stable angina but is rarely fatal. In contrast, thrombosis plays a major role in the pathogenesis of the life-threatening acute coronary syndromes (ACS), including ST-segment elevation myocardial infarction (STEMI), non-STEMI, and unstable angina—the latter, in particular, if acute chest pain occurs at rest.[6,7] Another common presentation of atherothrombosis is sudden coronary death.[8,9] Rare non-atherosclerotic causes of ACS include coronary arteritis, trauma, dissection, thromboembolism, congenital anomalies, cocaine abuse, and complications of cardiac catheterization.[10]

In this review, we will try to explain how a chronic disease that evolves silently over decades (atherosclerosis) suddenly and often unexpectedly puts a patient's health and life at risk due to coronary thrombosis.

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Atherothrombosis

Atherosclerosis is a systemic, lipid-driven immune-inflammatory disease of medium-sized and large arteries leading to multifocal plaque development,[11–14] predominantly at predilection sites characterized by low and oscillatory endothelial shear stress.[15,16] The disease begins to develop early in life but the speed of progression varies greatly and is difficult to predict. However, it usually takes decades to develop the advanced lesions responsible for clinical disease, offering unique opportunities for timely detection and personalized prevention. While most plaques remain asymptomatic (subclinical disease), some become obstructive (stable angina), and a few become thrombosis-prone (vulnerable) and may lead to an ACS. Although causal risk factors for atherothrombosis and CHD are well-known and constitute important therapeutic targets, their predictive value on a per-patient basis is limited,[17,18] and it remains a challenge to identify the apparently healthy individuals who are at high risk for the development of ACS event and need intensified prevention.

Plaques underlying coronary thrombi

It is much easier to explain an accident after it has occurred than to predict it. The same is true for coronary thrombosis. Therefore, let us first focus on the accident that did happen, the thrombosed coronary artery. Based on a review of the literature, including 22 autopsy studies in which 1847 coronary arteries were explored microscopically with the purpose of identifying the underlying cause of thrombosis, it can be concluded that the great majority of coronary thrombi (73%) developed on top of a ruptured atherosclerotic plaque (*Table 1*).[19–41]

Plaque rupture

Although pathologists appear to agree on what a ruptured plaque looks like in the microscope, the use of less well-defined terms such as disruption and fissuring have led to some confusion. While some investigators have used these terms synonymously,[6,42] others have not.[43,44] In a consensus statement, plaque rupture was defined as a structural defect—a gap—in the fibrous cap that separates the lipid-rich necrotic core of a plaque from the lumen of the artery (*Figure 1*).[45] Davies[46] used the terms fissure and rupture interchangeable and stressed that a variable mix of haemorrhage into the plaque and luminal thrombosis originating from the fissure/rupture site characterizes culprit lesions in ACS. In Virmani's experience, 'fissure' is defined as a lateral tear in an eccentric plaque with underlying small necrotic core. The superficial tear lifts a layer of the intima from the underlying fibrous tissue and the haemorrhage extends into the necrotic core and this tract is usually lined by macrophages. The lumen usually has a small

Table 1 Plaque rupture underlying 1345 (73%) of 1847 fatal coronary thrombi worldwide

Patients	Age,[a] years	Cases, *n*	Rupture, %	Study
Hospital, -	—	19	19/19 = 100	Chapman[19]
Hospital, -	—	17	17/17 = 100	Constantinides[20]
Hospital, AMI+SCD	58	40	39/40 = 98	Friedman and Van den Bovenkamp[21]
Hospital, AMI	62	88	71/88 = 81	Bouch and Montgomery[22]
Hospital, AMI	66	91	68/91 = 75	Sinapius[23]
Coroner, SCD	53	20	19/20 = 95	Friedman *et al.*[24]
Hospital, AMI	67	76	69/76 = 91	Horie *et al.*[25]
Hospital, AMI	67	49	40/49 = 82	Falk[26]
Coroner, SCD	<65	32	26/32 = 81	Tracy *et al.*[27]
Med. Exam, SCD	<70	61	39/61 = 64	El Fawal *et al.*[28]
Hospital, AMI	—	83	52/83 = 63	Yutani *et al.*[29]
Coroner, -	—	85	71/85 = 84	Richardson *et al.*[30]
Hospital, AMI	63	20	12/20 = 60	van der Wal *et al.*[31]
Coroner, SCD	—	202	143/202 = 71	Davies[32]
Hospital, AMI	69	291	218/291 = 75	Arbustini *et al.*[33]
Hospital, AMI	61	61	56/61 = 92	Shi *et al.*[34]
Hospital, AMI	69	100	81/100 = 81	Kojima *et al.*[35]
Med. Exam, SCD	48	360	212/360 = 59	Virmani *et al.*[36]
Med. Exam, AMI+SCD	—	31	26/31 = 84	Murai *et al.*[37]
Hospital, SCD	—	58	34/58 = 59	Giannoukas and co-workers[38]
Hospital, AMI	—	14	10/14 = 71	Sato *et al.*[39]
Coroner, SCD	54	49	23/49 = 47	Subirana *et al.*[40]
AMI + SCD	—	1847	1345/1847 = 73	Worldwide

Data from Virmani *et al.*[36] is updated by adding recent cases. Modified and reproduced with permission from Falk *et al.*, *J Am Coll Cardiol.*, 2006;47:C7–C12.
—, not reported; AMI, acute myocardial infarction; SCD, sudden coronary death.
[a]Mean.

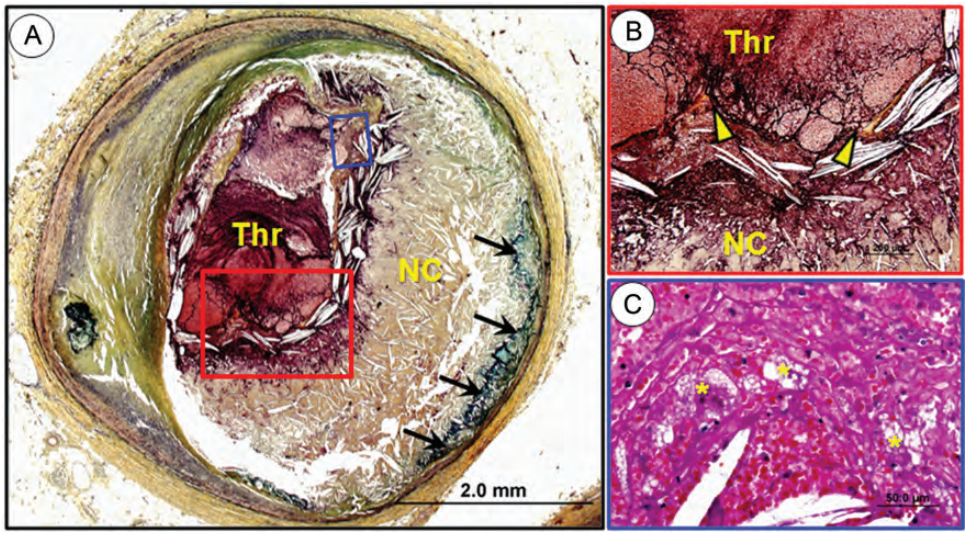

Figure 1 Plaque rupture. Cross-sectional photomicrograph of a coronary artery showing plaque rupture. (*A*) Note the presence of an acute occlusive luminal thrombus with an underlying large necrotic core (NC) and almost total absence of a fibrous cap. The medial wall is destroyed and near the base of 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).

difficult to appreciate thrombus. In our experience, this lesion is seen in 10–15% of sudden coronary death cases.

In our world-wide survey (*Table 1*), plaque rupture was the main cause of coronary thrombosis regardless of clinical presentation (MI: 79%; sudden coronary death: 65%), age (>60 years: 77%; <60 years: 64%; unknown: 73%), sex (men: 76%; women: 55%), and continent (Europe: 72%; USA: 68%; Asia 81%). The gender differences appears noteworthy. Recent clinical observations have confirmed that plaque rupture is the most common cause of coronary thrombosis not only in patients dying from the disease but also in those who survive.[47] Furthermore, clinically silent plaque rupture is not a rare phenomenon,[48,49] and plaque rupture with mural thrombosis appears to be a common cause of episodic but asymptomatic progression to severe stenosis.[50,51] These pathoanatomical findings explain classical clinical observations,[52–55] indicating that progression of atherosclerosis involves two distinct processes: a chronic one that leads to luminal narrowing slowly (atherosclerosis), and an acute one that causes rapid luminal obstruction (plaque haemorrhage and/or luminal thrombosis).

Plaque erosion (thrombosis without plaque rupture or calcified nodule)

As *Table 1* indicates, a ruptured plaque is not found beneath all coronary thrombi. However, it was not until the 1990's that the generic term 'plaque erosion' was introduced for thrombosis without plaque rupture.[31,56] Plaque erosion is 'identified' when serial sectioning of the thrombosed arterial segment fails to reveal plaque rupture (*Figure 2*).[36] Typically, the endothelium is missing at the erosion site, and the exposed intima consists predominantly of vascular smooth muscle cells and proteoglycans. The underlying plaque morphology shows presence of pathological intimal thickening or a fibroatheroma with an intact media, whereas in ruptured plaques the media is often destroyed.

Observations by Virmani *et al.*[36] indicate that the eroded site is minimally inflamed, but not all agree on that.[31,57] While Farb *et al.*[56] reported that, on average, eroded plaques with thrombosis are less obstructive than ruptured plaques with thrombosis, Kojima *et al.*[35] found no association between the degree of stenosis and the type of thrombosis, and Davies[4] found an association opposite to that reported by Farb *et al.* Sato *et al.*[39] observed that asymptomatic coronary thrombi usually were small and related to non-obstructive plaques with erosion rather than thrombi derived from plaque rupture while in patients dying of AMI the degree of underlying luminal narrowing and the size of coronary thrombi are significantly larger but equivalent between erosion and rupture. However, we must look at population being studied. Kojima *et al.* studied only patients presenting with acute MI (AMI). Davies *et al.* also studied patients dying suddenly but may have had known coronary heart disease. Virmani *et al.* studied patients dying of sudden coronary death but never having had any previous known heart disease. Also, the age of the patient population is significantly different: 49 ± 10 years by Virmani, 69 ± 10 years by Kojima, and those from Davies studies varied from 37 to 69 years but no mean age is available. Clinical studies of optical coherence tomography (OCT) performed in patients presenting with AMI showed that the incidence of fibrous cap disruption was 73%, whereas plaque erosion was 23% and patients with plaque rupture had a higher incidence of thin cap fibroatheroma 83%;[47] results were very similar to autopsy study published by Arbustini *et al.* in hospital patients dying with STEMI.[33] Recent data

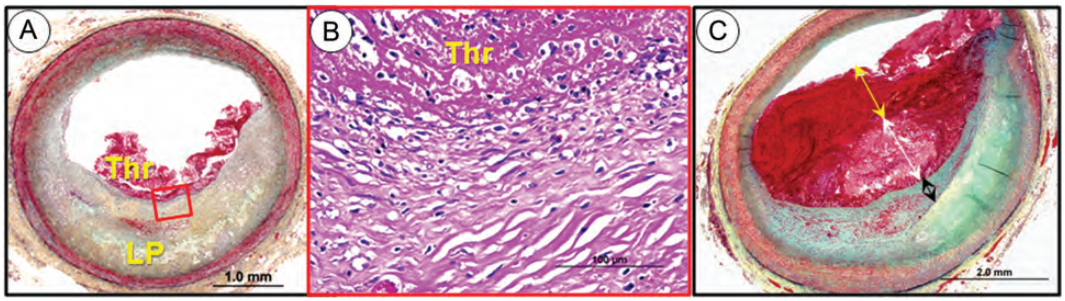

Figure 2 Plaque erosion. Cross-section of a coronary artery showing plaque erosion. (*A*) A non-occlusive thrombus (Thr) is present on the surface of a plaque which consists of pathological intimal thickening, note the artery is insignificantly narrowed. There is no connection between thrombus and the lipid pool (LP) and the media is intact. (*B*) Higher-magnification image of the red box in (*A*). Note the presence of thrombus and the under lying plaque which consists of smooth muscle cells in a proteoglycan–collagen rich matrix and there is absence of inflammation. (*C*) Shows another case of plaque erosion. The oldest layer of the plaque (black double arrow) is an organizing thrombus and is being replaced by smooth muscle cells in a proteoglycan-rich matrix and there is an overlying acute thrombus present in the lumen of varying ages (white and yellow double arrows).

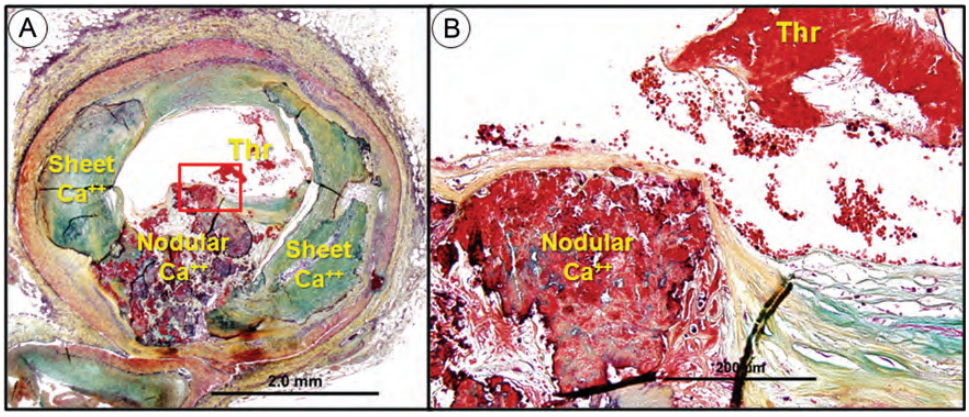

Figure 3 Calcified nodule. Cross-section of the coronary artery showing the presence of calcified nodule. Nodular calcifications (Ca^{++}) are identified between sheets of calcification. (*B*) Higher-magnification image of the red box in (*A*). The nodular calcification is protruding into the lumen area with an overlying non-occlusive thrombus.

(A.V. Finn *et al.*, in preparation) also suggest that the distinction between thrombosis caused by eroded vs. ruptured plaques may have therapeutic implications.

The authors of this review agree on the frequency of plaque rupture beneath coronary thrombi and consequently also on the complementary frequency of thrombi not caused by plaque rupture. However, a key question is whether the latter is a homogenous group that deserves the specific name 'erosion,' meaning that the endothelium is missing and implying that the missing endothelium plays a critical role in the development of coronary thrombosis on non-ruptured plaques. Falk and coworkers find it counterproductive to use a plaque-specific term for acute atherothrombotic events that may be precipitated by a systemic prothrombotic state and/or local flow disturbances rather than by missing endothelium. On the other hand, the missing endothelium may be the result of vasospasm a condition that cannot be diagnosed at autopsy, and the vessels show negative remodelling.

Calcified nodule

In 2000, the term 'calcified nodule' was introduced by Virmani *et al.* for a rare type of coronary thrombosis not caused by plaque rupture but related to disruptive nodular calcifications protruding into the lumen (*Figure 3*).[36] These occur usually in older individuals and in tortuous heavily calcified arteries. Calcified nodules have distinct features identifiable by intravascular ultrasound (irregular and convex luminal surface) permitting their identification *in vivo*.[58] Surprisingly, calcified nodules identified by intravascular ultrasound in PROSPECT (Providing Regional Observations to

Study Predictors of Events in the Coronary Tree) were unlikely to cause coronary events during 3-year follow-up.[59]

Risk factors and type of thrombosis

Does any relationship exist between the traditional risk factors and the mechanism behind the final thrombotic occlusion of a coronary artery? Except for sex and menopause, the short answer is no, not consistently. Here follows a summary of a more detailed review.[60]

Plaque rupture is a more common cause of coronary thrombosis in men (~80%) than in women (~60%),[26,33] and plaque rupture is especially rare in pre-menopausal women.[61] Regarding lipids, Burke *et al.* found a statistically significant association between thrombosis caused by plaque rupture (vs. erosion) and high total cholesterol (TC), low high-density lipoprotein cholesterol (HDL-C), and high TC/HDL-C ratio in men.[9] In women, only TC correlated with plaque rupture.[61] However, Kojima *et al.* found no relationship between TC and plaque rupture,[35] and rupture of a lipid-rich plaque is in fact the standard cause of coronary thrombosis in China (>90% of all cases), a population with a low average TC level.[34] Smoking seems to promote thrombosis rather than atherosclerosis,[9,62] and only few and inconsistent data exist on a possible relationship between smoking and type of thrombosis. Burke *et al.* found a relatively high frequency of smokers in pre-menopausal females with plaque erosion,[61] but no association in men,[9] and Kojima *et al.* reported that smoking was associated with plaque rupture.[35] For diabetes and/or glycosylated haemoglobin, contrasting results have been reported. Burke and Kojima found no association to the type of thrombosis,[9,35] but Davies observed that diabetes was associated with plaque erosion.[4,32] Hypertension does not appear to favour any particular type of thrombosis.[9,26,35,61] Regarding circulating biomarkers of inflammation, Burke *et al.* found no relationship between C-reactive protein measured post-mortem and the type of thrombosis,[62] confirmed recently *in vivo* by OCT in patients with ACS.[63] On the other hand, another circulating inflammatory biomarker, myeloperoxidase (MPO) was higher in patients with OCT-defined plaque erosion than rupture, and the density of MPO-positive cells was higher within thrombi overlying eroded (vs. ruptured) plaques in fatal coronary thrombosis.[63] Other biomarker that has recently been also implicated and are associated with the presence of multiple complex lesion morphology include neopterin, a pteridine derivative that is secreted by macrophages after stimulation by interferon γ.[64] Similarly, pregnancy-associated plasma protein-A (PAPP-A), a zinc- binding metalloproteinase, which has been reported to be abundantly expressed in ruptured unstable plaques and also in angiographic complex plaques.[65]

Plaques leading to coronary thrombosis: vulnerable plaques

Knowing how a thrombosed coronary artery looks in the microscope, it is possible to infer what the underlying plaque looked like just before the thrombus evolved. The term vulnerable plaque has been used for such plaques assumed to be at high risk of thrombosis.[45] As mentioned earlier, calcified nodules do not seem to be high-risk lesions,[59] leaving two major types of vulnerable plaques, the rupture-prone and the erosion-prone. They are presumed to look like the corresponding thrombosed plaques, just with preserved surface without thrombosis.

Rupture-prone plaques

The prototype of a presumed rupture-prone plaque contains a large and soft lipid-rich necrotic core covered by a thin and inflamed fibrous cap.[42,66] Associated features include big plaque size, expansive remodelling mitigating luminal obstruction (mild stenosis by angiography), neovascularization (angiogenesis), plaque haemorrhage, adventitial inflammation, and a 'spotty' pattern of calcifications (*Table 2*). Although the macrophage density in ruptured fibrous caps is high,[41,66] whole-plaque macrophage density rarely exceeds a few percent because ruptured caps are tiny.[67,68]

Erosion-prone plaques

Vulnerable plaques of the erosion-prone type are heterogeneous and defined only by their fate (thrombosis, mostly mural).[36] The surface endothelium is missing, but whether it vanished before or after thrombosis remains unknown. No single morphological features have been identified but, in general, eroded plaques with thrombosis are scarcely calcified, rarely associated with expansive remodelling, and only sparsely inflamed.[36] Thus, it remains a challenge to distinguish erosion-prone plaques from stable plaques by imaging.[69] However, recent clinical imaging studies by OCT have confirmed the presence of plaque erosion based on patients with aspiration of thrombi with absence of discontinuation of fibrous cap.[70,71] We still do not really understand the specific plaque features which specifically distinguish plaques prone to erosion from more stable plaque types.

Location and natural history

Vulnerable plaques, plaque rupture, and thrombosed plaques tend to cluster in 'hot spots' within the proximal segments of the major

Table 2 Features of ruptured plaques[a]

Thrombus
Large necrotic core (>30% of plaque)
Fibrous cap covering the necrotic core
Thin (thickness usually <65 μm)
Many macrophages (inflammation)
Few smooth muscle cells (apoptosis)
Expansive remodelling preserving the lumen
Neovascularization from vasa vasorum
Plaque haemorrhage
Adventitial/perivascular inflammation
'Spotty' calcification

[a]The same features, except rupture of the cap and luminal thrombus, characterize vulnerable plaques of the rupture-prone type.

coronary arteries,[72–74] and rarely more than one or a few such lesions exist simultaneously.[26,75] The natural history of vulnerable plaques such as the speed of development, lifetime (persistency) and fate, is, however, unknown.

Structural determinants of plaque rupture

Plaque rupture, the most common cause of coronary thrombosis, requires the presence of a lipid-rich necrotic core covered by a thin fibrous cap. The size of the necrotic core and the thickness of the fibrous cap appear to be the two major structural determinants of vulnerability, along with macrophage infiltration of the fibrous cap.

Necrotic core

During atherogenesis, the atherogenic lipoproteins are retained within the intima, modified and accumulate predominantly deeply in the abluminal part of the intima.[13,76,77] Some of these 'pools' of lipids seem to attract macrophages that secrete proteolytic enzymes and engulf lipid until they die, leaving behind a soft and destabilizing lipid-rich cavity containing cholesterol crystals and devoid of supporting collagen and cells, the 'necrotic core.'[36,42,78] Such a plaque is called an 'atheroma' or 'fibroatheroma.'[36,43] Recent evidence suggests that macrophage apoptosis coupled with defective phagocytic clearance of the apoptotic cells (efferocytosis) promotes plaque necrosis,[12] and extravasation of erythrocytes into the necrotic core may expand it (see 'Intraplaque haemorrhage' section subsequently).

Fibrous cap

The fibrocellular part of the plaque located between the necrotic core and the lumen is called the 'fibrous cap.' It is extremely thin in coronary plaque rupture.[42,66] Assessed by microscopic examination post-mortem, ruptured caps were usually <65 μm thick.[36] Assessed by OCT *in vivo*, the mean thickness was only 49 μm.[47] If the fibrous cap is thin, the plaque is called a 'thin-cap fibroatheroma' (TCFA).[36] In TCFA, the necrotic core occupies ~23% of plaque area.[66] The amount of inflammation varies, but culprit lesions in ACS are usually more inflamed than those in stable angina,[79,80] and the thin and disrupted fibrous caps are usually heavily inflamed (macrophage density ~26%).[66] Although the ability of a thin fibrous cap to accommodate macrophages is limited,[68] pro-inflammatory macrophages within the cap could play a key role in its degradation and ultimate rupture by secreting proteolytic enzymes such as matrix metalloproteinases.[5,81] Mast cells also have the potential to promote degradation of the fibrous cap.[82] Apoptosis is common at the site of fibrous cap rupture, usually confined to macrophages because the vascular smooth muscle cells (SMCs) already have vanished when rupture occurs.[83,84] With their ability to synthesize extracellular matrix, including collagen, loss of SMC is associated with impaired healing and repair, increasing the risk of plaque rupture.[85]

Atherosclerosis is an innate inflammatory disease in which smoldering inflammatory activity is not confined to just a few atherosclerotic lesions but is present, more or less, in all such lesions throughout the body.[86,87] In contrast, vulnerable plaques are relatively rare,[76] and inflammation may play a causal role in plaque rupture only if located within a thin fibrous cap, i.e. the microstructure of the plaque needs to be permissive for rupture. Thus, although plaque inflammation may be useful as a marker of disease activity, it is probably not useful as a stand-alone marker for plaque vulnerability.[88]

It has been reported that the two major determinants of plaque vulnerability, core size and cap thickness, are unrelated statistically.[89] Furthermore, neither of these two factors alone confers vulnerability and are not related to absolute plaque size or to the degree of stenosis.

Intraplaque haemorrhage

Plaque haemorrhage has for decades been recognized as an important cause of rapid plaque progression,[26] but not until recently was the focus shifted to plaque neovascularization (angiogenesis) and its role in intraplaque haemorrhage and the development of a vulnerable (rupture-prone) plaque.[90] To understand the background for the current way of thinking, it is appropriate to recapitulate Michael Davies' view on the role of plaque haemorrhage in the pathogenesis of sudden coronary death.[8] He concluded that 'we have avoided the term "plaque hemorrhage," since it is a source of confusion. "Plaque fissuring" is the term applied to the formation of an opening from the lumen into the intima; it leads to what was known originally as "dissecting hemorrhage" but is actually an intraintimal thrombus containing not just red cells but mainly fibrin and platelets. Pure plaque haemorrhage is defined as the presence of red cells within a plaque and is derived from small capillaries crossing into the intima from the media. Plaque fissuring is an important process; pure plaque haemorrhage was so universal in both test and control hearts that we have ignored it.'[8]

The question is whether we should ignore angiogenesis-derived plaque haemorrhage because it is universal or, for the same reason, explore it. We favour the latter. Recent evidence indicates that plaque haemorrhage may play an important role in rapid progression of atherosclerosis and, in particular, may contribute to necrotic core expansion and plaque vulnerability.[90] However, as stressed by Davies and others,[26] plaque haemorrhages do not always originate from plaque neovascularization (angiogenesis), some of the largest originate in fact from the lumen (plaque rupture), and this distinction is not just academic but may have important prognostic and therapeutic implications. As illustrated in *Figure 4*, it is not always easy to identify the origin of a plaque haemorrhage. We as authors of this joint manuscript do not always have similar opinions as in the case of plaque haemorrhage—vasa vasorum playing an important role is the dominant view in the literatures and by Virmani *et al.*; however, Falk *et al.*, similar to Davies, believes plaque rupture/fissure may be more important. Regardless of the origin of the haemorrhage, we can all agree that the occurrence of substantial plaque haemorrhage is an important event in the life of a plaque. It results in sudden enlargement of the plaque size resulting in luminal narrowing and is a

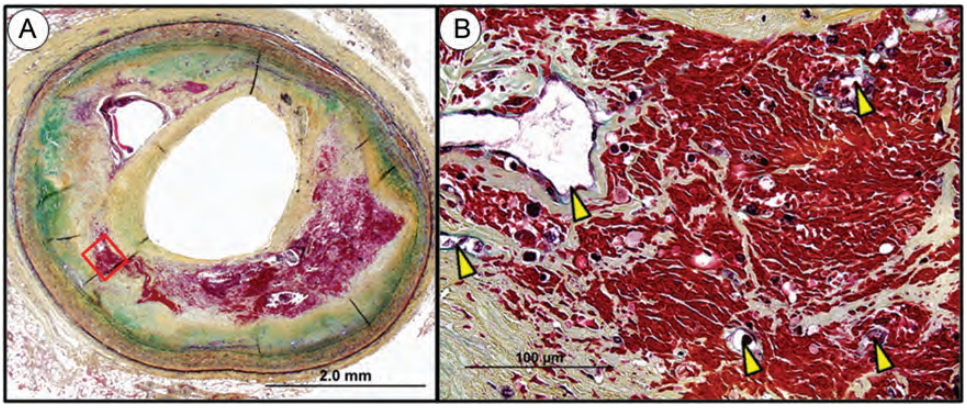

Figure 4 Intraplaque haemorrhage. Cross-section of a coronary artery showing haemorrhage into a plaque (necrotic cores) with severe narrowing of the lumen. (*B*) Higher-magnification of the red box area in (*A*). Note the presence of a number of small capillaries of varying sizes (arrowheads) in the area of haemorrhage. However, the mere presence of neovascularization does not prove that these haemorrhages originate from these fragile low-pressure microvessels within the plaque. Another possibility is a high-pressure haemorrhage from the lumen of the artery into the plaque via a ruptured fibrous cap not revealed in this particular cross-section of the lesion.

very important source of free cholesterol from the red cell membranes, which are rich in free cholesterol and cholesterol esters.[90,91]

Angiogenesis and inflammation often coexist at the base of advanced plaques.[92] The new microvessels rarely originate from the lumen but usually from vasa vasorum in adventitia.[93,94] They lack supporting cells and are fragile and leaky, giving rise to local extravasation of plasma proteins and erythrocytes.[95–97] Such intraplaque bleedings are common[8] and may expand the necrotic core, causing rapid progression of the lesion.[90] Another common source of plaque haemorrhage is extravasation of blood through a ruptured fibrous cap,[26] called an intraintimal thrombus by Davies.[8] An unresolved and interesting question involves whether haemorrhage itself either helps to resolve neovascularization or simply promotes it further.

Macrophage polarization

Macrophages play a very important role in the progression of atherosclerosis along with other cells like the T-cells and smooth muscle cells.[98] It is now well recognized that at least two if not three types of macrophage subtypes can be observed in atherosclerotic plaques. The most common macrophages in the atherosclerotic plaques are the classically activated macrophages M1, which are induced by INF-γ or other T helper 1 (Th1) cytokines, and trigger a pro-inflammatory response. Within the atherosclerotic plaque, these M1 cells show MHC class II expression and are foamy in nature, i.e. have a high lipid intake. The alternative M2 macrophages are activated by a different pathway through T helper 2 (Th2) cytokines, i.e. interleukin-4 (IL- 4) and IL-13. Bouhlel *et al.*,[99] described the presence of this type of macrophages (M2) in atherosclerotic plaques and reported that PPARγ controlled the M2 differentiation, which results in anti-inflammatory activity within the plaque. Boyle *et al.*[100] and our own group demonstrated that another type of macrophages exists in the plaque and occurs at the sites of haemorrhage or angiogenesis (*Figure 5*). We have shown that these haemoglobin-stimulated macrophages (M(Hb)) express both CD163 and mannose receptors and are devoid of neutral fats, which is typical of the foamy macrophages. The Hb macrophages in the plaque show the absence of CD36 expression (i.e. scavenger receptors) and instead have a high expression of ATP-binding cassette transporters.[98,100] They do not demonstrate the presence of pro-inflammatory cytokines such as TNF-α and have reduced the production of inducible nitric oxide synthase (iNOS). A decrease in intracellular iron likely plays a pivotal role in driving the transcription of genes which protect these cells from lipid accumulation in part by reducing intracellular iron-driven production of reactive oxygen species such as hydroxyl radical (OH°) through the up-regulation of ferroportin.

Plaque size, luminal obstruction, and remodelling

During atherogenesis, the artery tends to remodel in such a way that the luminal obstruction caused by some plaques is attenuated (expansive remodelling) and by others accentuated (constrictive remodelling). Although vulnerable plaques of the rupture-prone type (TCFA) are usually big,[101] they often are invisible or appear non-obstructive by angiography because of compensatory expansive remodelling and/or extension of the plaque to the adjacent reference segments judged to be normal by angiography.[102,103] In contrast, plaques responsible for stable angina usually are smaller but, nevertheless, often associated with more severe luminal narrowing by angiography because of concomitant constrictive remodelling.[103] The reasons for the different modes of remodelling remain to be defined, but recent clinical observations indicate that diabetes is accompanied by inadequate compensatory remodelling.[104]

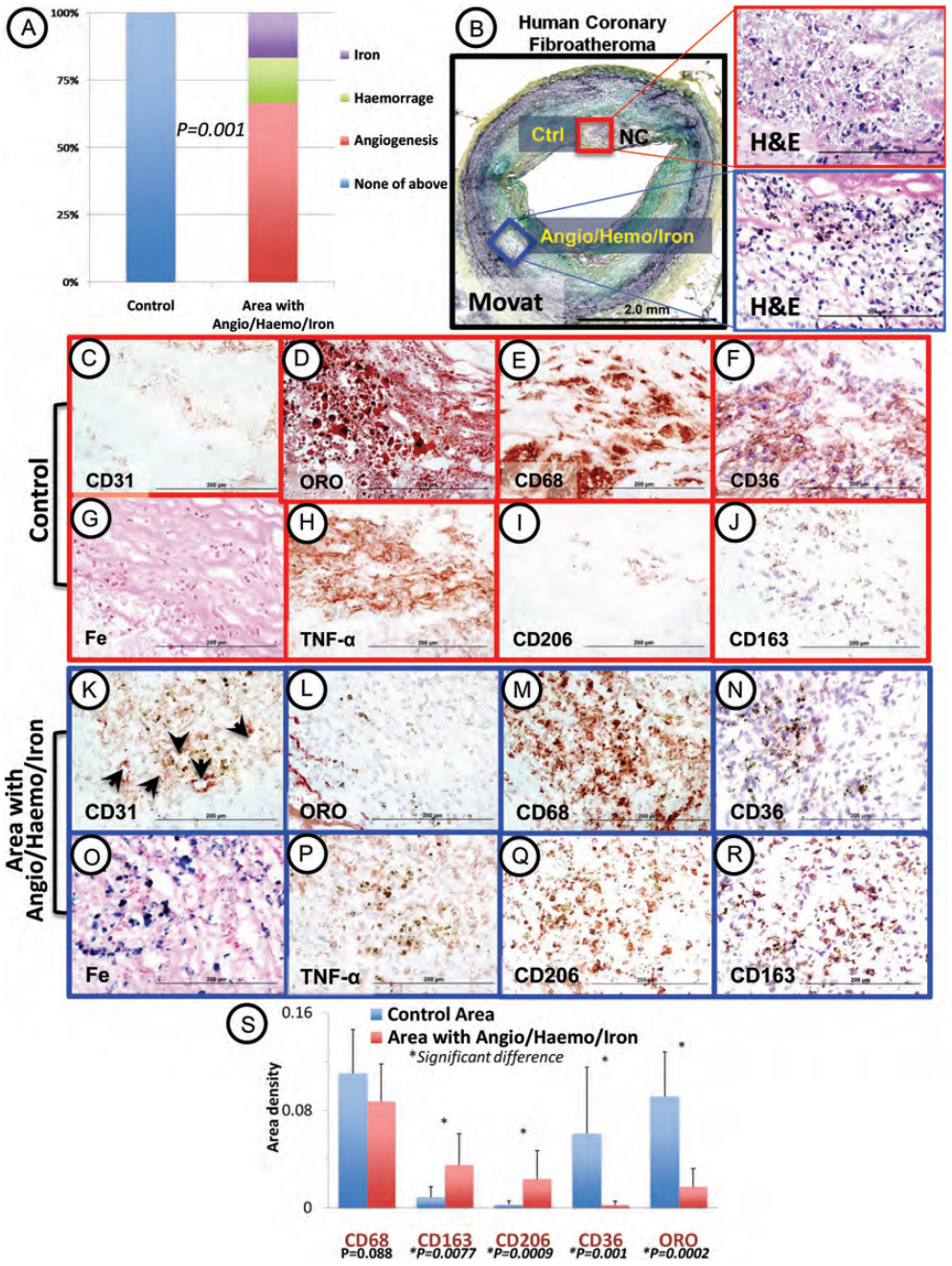

Figure 5 M2 markers CD206/CD163 are preset in human atherosclerotic lesions at the site of prior haemorrhage and are distinct from foam cells. (*A*) Plaque regions were identified by the presence of angiogenesis/haemorrhage/iron (Angio/Haemo/Iron) and compared with control (Ctrl) pericore regions of macrophages devoid of Angio/Haemo/Iron. (*B*) Representative frozen section of human coronary fibroatheroma from a 48-year-old man who died suddenly. High-power image from Ctrl (red box) shows foamy macrophages in the perinecrotic core (NC) region. The blue box shows an area rich in angiogenesis and iron. Low-power image, Movat stain; high-power images, haematoxylin and eosin (H&E) stain. Photomicrographs of the boxed areas from Ctrl (red boxes) (*C–J*) and area of Angio/Haemo/Iron (blue boxes) (*K–R*). Note that the Ctrl area shows a lack of CD31 staining (brown) (*C*), abundant oil red O (ORO) positivity (red) (*D*), macrophage infiltration (CD68, brown) (*E*), CD36 staining (brown) (*F*), and no iron staining (blue, Perl Prussian blue) (*G*). It also demonstrates abundant tumour necrosis factor α (TNF-α) positivity (brown) (*H*), but there is minimal MR (CD206, brown) (*I*) and CD163 (brown) (*J*) immunostaining. (*K–R*) are from an area of Angio/Haemo/Iron showing abundant CD31 staining (*K*, black arrows point to angiogenesis), rare positive cells for oil red O (*L*), but abundant C68 staining (*M*). This area also demonstrates minimal CD36 staining (*N*), but abundance of iron (*O*), minimal TNF-α staining (*P*), but positive staining for CD206 and CD163 (*Q* and *R*). Quantitative analysis (*S*) from Ctrl and Angio/Haemo/Iron areas from 14 plaques demonstrated equivalent macrophage area density (CD68) but higher expression of CD163 and CD206 in regions of Angio/Haemo/Iron than in Ctrl regions. Scale bars: low-power, 2 mm; high-power: 200 μm (non-normal distribution CD68 and CD163). Fe = iron. (Reproduced with permission from Finn *et al.*[98])

Calcification

Focal calcifications in atherosclerotic plaques are very common and increase with age.[105] At autopsy, the amount of coronary artery calcium (CAC) correlates only modesty with luminal narrowing but more strongly with plaque burden.[106] Apoptotic cells, extracellular matrix, and necrotic cores may calcify, healed ruptured plaques are often heavily calcified, and microcalcifications have been described in the fibrous cap.[107,108] The pathogenesis and clinical significance of these different forms of calcifications are, however, poorly understood. Non-atherosclerotic calcifications of coronary arteries are exceedingly rare except in chronic renal failure where Mönckeberg medial calcific sclerosis may be seen.[109]

Clinical observations suggest that culprit lesions responsible for ACSs generally are less calcified than plaques responsible for stable angina, indicating that calcium confers stability to plaques rather than the opposite.[110] However, the pattern of plaque calcification may also matter; a 'spotty' (vs. dense) pattern is more common in high-risk vs. lower-risk plaques.[111–113] The total amount of calcification—the CAC score—is a marker of plaque burden (and thus a marker of cardiovascular risk) rather than a marker of risk conferred by the individual plaque.[114] The questionable predictive value of calcified nodules has already been discussed, and the clinical usefulness of the CAC score in risk stratification will be discussed at the end of this review.

Prospective detection of vulnerable plaques

PROSPECT (Providing Regional Observations to Study Predictors of Events in the Coronary Tree) was the first and is the largest natural-history study of coronary atherosclerosis using multimodality intracoronary imaging to identify vulnerable plaques to date.[115] Assessed by angiography, most new coronary events originated from non-obstructive lesions. By intravascular ultrasound, larger plaque burden (>70% cross-sectional area narrowing) and smaller minimal luminal area (<4.0 mm^2) and the presence of a virtual histology (VH)-defined TCFA were independently associated with an increased risk for subsequent events. Those few lesions that possessed all three characteristics had an 18.2% rate of major coronary events during the 3.4-year follow-up period. However, the predictive power of VH-defined TCFA alone was low, mainly because of poor specificity. Of 595 TCFAs identified by VH, only 26 led to coronary events during follow-up. This disappointing result may not necessarily mean that the vulnerable plaque concept is flawed, it may rather indicate that the tools used to prove the concept are inadequate to correctively identify high-risk plaques.[116–118] Further prospective study of this issue is warranted as newer more sensitive imaging technologies become available.

Onset of ACS: vulnerability vs. triggers

Sudden rupture of a thin and inflamed fibrous cap may occur spontaneously but triggering could also play a role and thus help explaining the non-random onset of ACS. As reviewed recently, many studies have identified a transiently increased risk of ACS during or immediately after short-term exposure to 'acute risk factors' such as physical and sexual activity, anger, anxiety, work stress, earthquakes, war and terror attacks, temperature changes, infections, and cocaine use.[119] The triggering pathways may include activation of the sympathetic nervous system with transient increases in blood pressure, heart rate, platelet activity, and arrhythmias, leading to plaque rupture, thrombosis, and/or sudden death in susceptible individuals. The absolute risk of an acute cardiovascular event depends on the baseline risk that increases with the number of risk factors and evidence of pre-existing cardiovascular disease. In this context, it is important to stress that regular physical activity (fitness) is associated with a lower baseline risk.

Although the relative risk of ACS during or immediately after a triggering activity may seem substantial, the absolute risk is usually small in individuals without known cardiovascular disease.[119] For instance, in a population-based study in which vigorous physical exertion was associated with a six-fold increased risk of MI, this led to an average of only 1.5 excess events per million hours of physical activity.[120] We have previously provided autopsy evidence for a link between vulnerable plaques in the coronary arteries and exertion-induced plaque rupture in sudden coronary death.[121] However, exercise stress testing in patients with advanced coronary atherosclerosis rarely triggers an ACS, suggesting that potential stressors may trigger events only among the relatively few susceptible individuals.

Clinical presentation: dynamic thrombosis and collaterals

The culprit lesion in ACS is frequently 'dynamic,' causing intermittent flow obstruction,[122–126] and the clinical presentation and the outcome depend on the location of the obstruction and the severity and duration of myocardial ischaemia.

A non-occlusive or transiently occlusive thrombus most frequently underlies ACS without ST-segment elevation, whereas a more stable and occlusive thrombus prevails in STEMI—overall modified by vascular tone and collateral flow.[127,128] A critical thrombotic component is also frequent in culprit lesions responsible for out–of–hospital cardiac arrest and sudden coronary death.[8,9] There are three major determinants of the thrombotic response to plaque rupture: the local thrombogenic substrate, local flow disturbances, and the systemic thrombotic propensity—also called 'Virchow's triad.'[129]

In plaque rupture, the exposed necrotic core appears to be very thrombogenic,[130] most likely due to tissue factor and prothrombotic microparticles left behind after apoptotic cell death.[131,132] In contrast to venous thrombosis, rapid flow and high shear forces are more important and promote thrombosis via shear-induced platelet activation.[133] A platelet-rich thrombus may indeed form and grow within a severe stenosis, where the blood velocity and shear forces are highest.[123] Finally, the state (activation) of platelets, coagulation, and fibrinolysis is critical for the outcome of plaque disruption, documented by the protective effect of

antiplatelet agents and anticoagulants in patients at risk of coronary thrombosis. In ACS, circulating tissue factor and prothrombotic microparticles could be critical for the thrombotic response to plaque disruption.[132]

Platelets, fibrin, and thrombotic burden

In coronary thrombosis, the initial flow obstruction is usually caused by platelet aggregation, but fibrin is important for the subsequent stabilization of the early and fragile platelet thrombus. Thus, both platelets and fibrin are involved in the evolution of a stable and persisting coronary thrombus.[123] If the platelet-rich thrombus (white macroscopically) at the site of plaque disruption occludes the lumen totally, the blood proximal and distal to the occlusion will stagnate and may coagulate, giving rise to a secondarily formed venous-type stagnation thrombosis (red macroscopically).[129] Stagnation thrombosis may contribute significantly to the overall thrombotic burden, particularly in occluded vein grafts (no side branches), and thus hamper recanalization.[134] Clinical experiences indicate that it is indeed very difficult to recanalize an occluded vein graft rapidly by intravenous thrombolytic therapy alone.

Dynamic thrombosis and microembolization

The thrombotic response to plaque rupture is dynamic: promoting a dynamic interplay between prothrombotic and prothrombolytic processes, and often associated with vasospasm.[135] Both occur simultaneously, causing intermittent flow obstruction, with partial thrombus dissolution and distal embolization.[122,136] The latter leads to microvascular obstruction, which may prevent myocardial reperfusion despite a 'successfully' recanalized infarct-related artery. Microvascular obstruction has been reported to be more frequent in erosion than in rupture in individuals presenting with sudden coronary death who have never been instrumented.[137]

Primary prevention

Causal risk factors for CHD constitute important therapeutic targets, but their usefulness as predictors with any specificity for disease-related events is quite limited.[17,18] Most ACS occurs in people at average risk-factor level who are misclassified by traditional risk factor scoring, as low or intermediate risk.[138,139] Conversely, others are misclassified as high risk and advised to take drugs to reduce their risk factor(s). These facts of daily clinical practice remind us that, although exposure to causal factors is important, susceptibility to these factors and the disease in question might be even more important and complex. Despite great promise, genetic testing for susceptibility has not proven useful for risk stratification.[140,141]

Atherosclerosis develops silently over decades before symptoms eventually occur, offering unique opportunities for timely detection and personalized prevention. Subclinical atherosclerosis can be detected and quantified non-invasively, to show the cumulative effect of all risk and susceptibility factors combined—known and unknown.[142] Three measures of disease burden have proven useful for risk assessment in clinical practice: coronary artery calcium by computed tomography, intima–media thickness and plaque area on carotid ultrasound, and ankle–brachial index. The 2010 American College of Cardiology Foundation and American Heart Association's guidelines for cardiovascular risk assessment recommend (class IIa) the use of these non-invasive tests for subclinical atherosclerosis in asymptomatic adults at intermediate risk according to traditional risk-factor scoring.[143] Similarly, class IIa recommendations were included in the European Guidelines on cardiovascular disease prevention, version 2012.[144] These tests for subclinical (asymptomatic) atherosclerosis can correctly reclassify a substantial number of people in the therapeutic grey area called 'intermediate risk' to lower or higher risk categories, for which treatments are better defined.[145–148] In the near future, not only plaque burden but also disease activity and plaque vulnerability may be assessed by imaging,[88,112,149–152] with the potential to improve risk assessment further and thus limiting both undertreatment and overtreatment.

Conclusions

We have systematically discussed plaque morphology associated with ACS and have also discussed very briefly macrophage subtypes that are present in human atherosclerotic plaques. What is clear overall is that the main cause of coronary thrombosis is plaque rupture and that risk factors are not always predictive of the extent of atherosclerosis in an individual patient but are predictive in large population studies. There is no controversy about the frequency of thrombosis not caused by plaque rupture, so called plaque erosion, but disagreement exists on the role of missing endothelium in the pathogenesis of coronary thrombosis. What can be agreed upon is that thrombosis-prone (vulnerable) plaques are worth identifying in order to treat such lesions aggressively but not necessarily invasively. However, this is a very complicated area of research fraught with problems but may be greatly helped by the development of newer imaging modalities in the future.

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