Stabilisation of atherosclerotic plaques

Position Paper of the European Society of Cardiology (ESC) Working Group on Atherosclerosis and Vascular Biology

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Summary

Plaque rupture and subsequent thrombotic occlusion of the coronary artery account for as many as three quarters of myocardial infarctions. The concept of plaque stabilisation emerged about 20 years ago to explain the discrepancy between the reduction of cardiovascular events in patients receiving lipid lowering therapy and the small decrease seen in angiographic evaluation of atherosclerosis. Since then, the concept of a vulnerable plaque has received a lot of attention in basic and clinical research leading to a better understanding of the pathophysiology of the vulnerable plaque and acute coronary syndromes. From pathological and clinical observations, plaques that have recently ruptured have thin fibrous caps, large lipid cores, exhibit outward remodelling and invasion by vasa vasorum. Ruptured plaques are also focally inflamed and this may be a common denominator of the other pathological features. Plaques with similar characteristics, but which have not yet ruptured, are believed to be vulnerable to rupture. Experimental studies strongly support the validity of anti-inflammatory approaches to promote plaque stability. Unfortunately, reliable non-invasive methods for imaging and detection of such plaques are not yet readily available. There is a strong biological basis and supportive clinical evidence that low-dose lipoprotein lowering with statins is useful for the stabilisation of vulnerable plaques. There is also some clinical evidence for the usefulness of antiplatelet agents, beta blockers and renin-angiotensin-aldosterone system inhibitors for plaque stabilisation. Determining the causes of plaque rupture and designing diagnostics and interventions to prevent them are urgent priorities for current basic and clinical research in cardiovascular area.

Keywords

Atherosclerosis, imaging, plaque stabilisation, treatment, vulnerable plaques

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Introduction

Atherosclerosis is a systemic, lipid-driven inflammatory disease of the arterial wall leading to multifocal plaque development (1–3). The progression varies from person to person but it usually takes decades to develop advanced atherosclerotic lesions responsible for clinical symptoms. Most plaques remain asymptomatic (subclinical disease), some become obstructive (stable angina) but a few become thrombosis-prone (vulnerable) and lead to atherothrombotic events, such as myocardial infarction (MI) and stroke (3–5). Despite a minority, vulnerable plaques in coronary and carotid arteries are the main cause of death and disability (6, 7).

The concept of plaque stabilisation was introduced several years ago to explain how acute coronary events could be reduced by lipid-lowering therapy without concomitant regression in coronary atherosclerosis assessed by angiography (8). It is now well-established that the risk of thrombosis depends more on plaque composition than on the degree of luminal obstruction seen by angiography. Therefore, a plaque may be stabilised against thrombosis independent of changes in plaque size and luminal obstruction. However, whether it will be possible to detect prospectively plaques at risk of thrombosis, target therapy to those lesions and eventually reduce the risk of clinical events, remain to be proven (9). The purpose of this position paper is to review key aspects of vulnerable plaques regarding their pathogenesis, detection and possibilities to stabilise these life-threatening lesions.
Vulnerable plaques

The great majority of symptomatic coronary thrombi (~75%) are caused by plaque rupture (5). Plaque rupture with mural thrombosis (+ plaque haemorrhage) is also a common cause of episodic but asymptomatic progression to severe stenosis (10, 11). The remaining thrombi are caused by less well-defined mechanisms of which the so-called “plaque erosion” is the most common type (5). Plaque rupture is a more frequent cause of coronary thrombosis in men (~80%) than in women (~60%) but, except for gender and menopause, no other risk factors have consistently been connected with a particular mechanism of thrombosis (3).

By inference, there are two major types of vulnerable plaques, rupture-prone and erosion-prone, that are presumed to resemble the corresponding thrombosed plaques, in the absence of rupture and thrombosis (5, 12, 13). The prototype of a rupture-prone plaque contains a large and soft lipid-rich necrotic core covered by a thin and inflamed fibrous cap (12–14). Associated features include large plaque size, expansive remodelling mitigating luminal obstruction (mild stenosis by angiography), neovascularisation, plaque haemorrhage, adventitial inflammation, and a “spotty” pattern of calcifications (Fig. 1) (3, 5). Although the macrophage density in ruptured caps is high, whole-plaque macrophage density rarely exceeds a few percents because ruptured caps are tiny (14).

Vulnerable plaques of the erosion-prone type are heterogeneous and defined only by their fate (thrombosis, mostly mural) (5, 15). The surface endothelium is missing, but whether it vanished before or after thrombosis remains unknown. No distinct morphological features have been identified (15) but, in general, eroded plaques with thrombosis are scarcely calcified, rarely associated with expansive remodelling, and only sparsely inflamed (15). So, irrespective of the plaque type, it is a misconception that vulnerable plaques are globally inflamed. Advanced plaques, including those that appear vulnerable, are in general hypocellular and consist mainly of fibrous tissue, necrosis, and calcifications (3, 16).

Vulnerable plaques, plaque rupture, and thrombosed plaques tend to cluster in “hot spots” within the proximal segments of the side branches of the major coronary arteries (17, 18), and rarely more than one or a few such lesions exist simultaneously (18, 19). The natural history of vulnerable plaques such as speed of development, lifetime (persistency) and fate is presently mostly unknown.

Coronary Plaque Rupture

1. Plaque size↑
2. Expansive remodelling
3. Necrotic core↑
   - ~54% of plaque area
   - ~3.8 mm², ~9 mm long
4. Fibrous cap
   - thickness: ~23 μm (95% ~65 μm)
   - macrophages (~7), ~26% of cap
   - smooth muscle cells (~ apoptosis)
   - thrombus
5. Angiogenesis↑
   - intraplaque haemorrhage
6. Perivascular inflammation
7. Calcification↓ - spotty

Figure 1: Coronary plaque rupture and rupture-prone vulnerable plaques. For comparison, a ruptured plaque with thrombus (top) and an intact and stable plaque (bottom) are depicted, and vulnerable plaque features are listed to the right. By inference, vulnerable plaques of the rupture-prone type are presumed to look like plaque rupture except for an intact cap.
Determinants of vulnerability

Plaque rupture 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 (Fig. 1).

Necrotic core

During atherogenesis, atherogenic lipoproteins are retained within intima, modified and accumulate, predominantly deeply in the abluminal part of intima (20, 21). Some of these „poools“ 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. Such a plaque is called an atheroma or fibroatheroma (15, 22).

Later on, plaque neovascularisation (angiogenesis) supervenes (23). The new microvessels rarely originate from the lumen but usually from vasa vasorum in adventitia (24). They lack supporting cells and are fragile and leaky, giving rise to local extravasation of plasma proteins and erythrocytes (23). Such intraplaque bleedings are common and may expand the necrotic core, causing rapid progression of the lesion (25). Another not uncommon source of plaque haemorrhage is extravasation of blood through a ruptured fibrous cap (19).

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. Assessed by microscopic examination postmortem, ruptured caps were usually <65 microns thick (14). Assessed by optical coherence tomography (OCT) in vivo, the mean thickness was only 49 microns (26). If the fibrous cap is thin, the plaque is called a thin-cap fibroatheroma (TCFA) (14). In TCFA, the necrotic core occupies ~23% of plaque area (14). Thin fibrous caps are usually heavily inflamed (macrophage density ~14%), particularly those that have ruptured (macrophage density ~26%), but because they are thin, their ability to accommodate macrophages is limited (14). Apoptosis is common at the site of fibrous cap rupture, usually confined to macrophages because vascular smooth muscle cells (SMCs) already have vanished when the rupture occurs (14, 27). With their ability to synthesise extracellular matrix, including collagen, SMC apoptosis is associated with impaired healing and repair, increasing the risk of plaque rupture.

Inflammation

Atherosclerosis is an 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. In contrast, vulnerable plaques are relatively rare, 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 the 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.

Expansive remodelling

During atherogenesis, the artery tends to remodel in such a way that the luminal obstruction is either attenuated (expansive remodelling) or accentuated (constrictive remodelling). Although vulnerable plaques of the rupture-prone type (TCFA) are usually large, they are often non-obstructive by angiography because of the expansive remodelling (28). It has been called the „remodelling paradox” because it is not good to preserve the lumen if it occurs at the expense of a higher risk of MI (29). 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 (29). The reasons for the different modes of remodelling remain to be elucidated, but recent clinical observations indicate that diabetes is accompanied by inadequate compensatory remodelling (30).

Are there animal models for vulnerable plaques?

Over the last 15 years, the mouse has become the predominant species to study experimental atherosclerosis, and a number of recent reviews have extensively discussed the various mouse models available (for example [31–33]). Most strains of mice are relatively resistant to the development of atherosclerosis due to a lipid profile significantly different from humans. Mice in the wild or on a rodent chow diet exhibit high plasma levels of atheroprotective high-density lipoprotein (HDL) and low concentrations of atherogenic low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL). Wild-type mice do not develop atherosclerosis unless challenged for long periods with high-fat, high-cholesterol diets (34). The current mouse models of atherosclerosis are therefore based on perturbations of lipoprotein metabolism through genetic manipulations.

The two most commonly used mouse models of atherosclerosis are the apolipoprotein E-deficient mouse (apoE−/−) (35, 36) and the LDL receptor-deficient mouse (LDLR−/−) (37). ApoE−/− mice are hypercholesterolaemic and spontaneously develop atherosclerotic lesions starting at early adulthood, even on a normal chow diet. LDLR−/− mice have a more modest lipoprotein abnormality than apoE−/− mice and development of atherosclerosis is very slow on a chow diet. Both mouse models rapidly develop atherosclerosis on a high-fat, high cholesterol diet. As in humans, atherosclerosis in mice develops in regions of the vasculature subjected to low or oscillatory wall shear stress (38). Predilection sites in the mouse are the aortic root, the lesser curvature of the aortic arch and branch points of the brachiocephalic, left carotid and subclavian arteries. Of note, retro-valvular lesions in the aortic root either stop abruptly at the orifice of the common coronary artery or extend a short distance into the arterial trunks but, in contrast to the human

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Inflammation and related production of proteases in vulnerable plaques

Inflammatory cells in atherosclerotic lesions

The fibrous caps of vulnerable plaques contain abundant blood-derived leukocytes, including monocytes, macrophages and T-lymphocytes. Of the T-cells CD4+ T-helper (Th) lymphocytes are the most prominent (50). Initially present in a Th0 ground state, after engagement of the T-cell receptor, naïve T-cells can differentiate to Th1 cells, which secrete and respond to interferon-γ (IFN-γ) or to Th2 cells, which secrete and respond to interleukin-4 (IL-4), IL-10 and IL-13 (Fig. 2). Interestingly, IFN-γ blocks Th2 differentiation while IL-4 blocks Th1 differentiation so there is a tendency for the T helper response to become polarised. Genetic manipulation of IFN-γ, IL-4 and their receptors in mice suggests that Th1 and, to a lesser extent, Th2 lymphocytes both promote atherosclerosis and histological features of vulnerability (51). By contrast, several populations of regulatory T-cells (Tregs) and their characteristic cytokines, including IL-10 and transforming growth factor (TGF)-β, consistently reduce atherosclerosis and favour stable plaque morphology (51). Thus, immunomodulation which aims to change the T-helper cell milieu and promote Tregs, seems an attractive possibility (50, 51).

T-cells promote plaque vulnerability locally through their effects on macrophages and foam-cell macrophages (FCMs), which are ultimately mostly derived from blood monocytes. Somewhat similar to the Th lymphocytes, there are several different macrophage phenotypes (Fig. 2). These include so-called classically-activated (or M1) and alternatively-activated (or M2) phenotypes together with more recently described regulatory phenotypes (52, 53). Classical activation by pro-inflammatory cytokines, including IFN-γ, amplifies production of pro-inflammatory mediators, MHC-II related antigens and extracellular proteases, thereby tending to promote inflammation and tissue destruction (52, 54). By contrast, alternative activation of macrophages with IL-4 or IL-13 suppresses production of pro-inflammatory cytokines, MHC-II related antigens and proteases, and increases secretion of connective tissue growth factors, thereby promoting granuloma formation and tissue repair (52). Regulatory macrophages that produce antioxidant enzymes and IL-10 are proposed to inhibit inflammation in a similar manner to Tregs. Indeed, anti-inflammatory cytokines, including IL-10 and TGF-β, suppress both classical and alternative activation of macrophages (Fig. 2).

FCMs can be found in atherosclerotic plaques and most FCMs are thought to be classically activated (50) but Bouhlel et al. (55) found macrophages expressing markers of alternative activation in human carotid plaques and noted a different distribution of these cells compared to macrophages with markers of classical activation. Boyle et al. showed that intra-plaque haemorrhage provokes accumulation of a ‘repair’ phenotype that may be responsible for clot resolution (56).
Modulation of macrophage phenotype

Based on the above knowledge it seems logical that interventions that deactiviate inflammatory macrophages, that favour M2 over M1 polarisation, or promote the formation of regulatory phenotypes should all improve plaque stability. For example, peroxisome proliferator activated receptor (PPAR) agonists and liver X receptor (LXR) agonists (57–59) can deactivate classically and alternatively activated macrophages in vitro and in vivo, and have shown beneficial effects on atherosclerosis in experimental models. Furthermore, macrophage-specific over-expression of LXRα greatly reduces atherosclerosis in mice, through increased cholesterol efflux, and possibly also through reduced classical activation (60). Conversely, gene knockout of PPARγ prevents alternative activation of macrophages in mice (61). PPARγ activation primes human monocytes to differentiate towards an anti-inflammatory phenotype that also expresses more PPARγ and could therefore participate in positive feedback (55). PPARγ activation was, however, unable to reverse classical activation or switch macrophages from classic to alternative activation programmes either in vitro or in plaques (55).

Inflammatory and anti-inflammatory cytokines

It is now increasingly appreciated that the immune response—besides being atheroprolressive—can be counterbalanced by atheroprotective cytokines. For instance, inhibition of TGF-β signalling using blocking antibodies accelerated atherosclerosis and induced a vulnerable plaque phenotype in mice (62). Disruption of TGF-β signalling in T-cells also increased atherosclerosis in mice, suggesting that TGF-β acts by attenuating T-cell activation (63). In human carotid atherosclerotic plaques, elevated expression of the TGF-β signalling pathway has been associated with higher collagen and SMC content and a more stable plaque phenotype (64). Moreover, atherosclerotic lesions of IL-10-deficient mice displayed abundant T-cell infiltration and IFN-γ expression but decreased collagen content, whereas transfer of murine IL-10 markedly reduced lesion size, highlighting a critical role of IL-10 in atherosclerotic lesion formation and stability (65).

Chemokines and growth factors

Chemokines are a large family of small related cytokines that regulate cell trafficking of leukocytes to areas of injury (51). To date, 42 chemokines and 18 chemokine receptors have been identified. Chemokines have been grouped into four subfamilies, CXC, CC, CX3C and C chemokines.

A set of independent studies has convincingly revealed that CCR5, the receptor for the platelet-derived chemokine RANTES/CCL5, drives a pro-inflammatory Th1-type immune response and supports advanced atherosclerotic plaque formation, whereas its deficiency is atheroprotective and confers features of plaque stability, an effect probably residing in bone marrow-derived cells (66, 67). Lesions with CCR5 deficiency showed a marked reduction in matrix metalloproteinase (MMP)-9 expression and increase in collagen accumulation (67). Fractalkine is expressed in murine and human lesions (68). CXCL1, the mouse orthologue of IL-8, and fractalkine/CX3CL1 were up-regulated beyond the initial stage and blocking fractalkine inhibited plaque growth and transformed vulnerable to stable plaques (69). Expression of IL-8 and IL-6 was also correlated with a vulnerable plaque phenotype in human carotid atherectomy specimen (70). This effect is probably mediated through a local activation of perivascular mast cells, as they increase vascular leakage, induce plaque haemorrhage, macrophage apoptosis and leukocyte infiltration through the IL-8 receptor CXCR2 (71).

Notably, the pleiotropic chemokine-like cytokine and CXCR2 agonist macrophage migration inhibitory factor (MIF) is up-regulated during atheroproggression, is abundantly present in advanced complicated lesions and can induce MMP-9 expression (72). Inhibition of MIF with a monoclonal antibody shifted the composition of vulnerable plaques towards a highly stable phenotype with a negligible FCM content and increased SMC/collagen content but also conferred a regression and stabilisation of primary atherosclerotic lesions in ApoE-/- mice, suggesting an interesting therapeutic option (73). In this context, it is noteworthy that MIF expression in human carotid atherectomy specimens has been identified as one of the most powerful predictors of systemic cardiovascular events (74). Another important mediator, monocyte chemotactic protein-1 (MCP-1/CCL2), is also expressed in atherosclerotic lesions, where it participates in monocyte recruitment (75). Beyond its importance in angiogenesis, vascular endothelial growth factor (VEGF) is expressed along with its receptors in atherosclerotic plaques (76) and may contribute to plaque progression and expansion of the vulnerable phenotype after systemic application (77–79), although short-term expression of VEGF does not enhance atherogenesis in LDLR-/- mice (80). Importantly, plaques in ApoE-/- mice show extremely high levels of inflammation owing to the exceedingly high levels of plasma cholesterol, which are only rarely found in cases of homozygous familial hypercholesterolaemia. Hence, care should be taken not to directly extrapolate mouse data to human pathology.

Extracellular proteases

Production of extracellular proteases including MMPs and cathepsins from macrophages promotes many of the adverse structural changes associated with plaque vulnerability. Loss of collagen in the plaque cap reduces tensile strength, while absence of collagen from the lipid core promotes transfer of hydrodynamic stress to the cap during the cardiac cycle. Both factors favour plaque rupture. Loss of elastin leads to outward remodelling, which is associated with plaque vulnerability and ultimately causes aneurysms. Extracellular proteases directly promote migration of macrophages and endothelial cells into plaques; they can also do this by cleaving cell surface and signalling molecules, such as growth factors and cytokines (81). Proteolysis of the extracellular matrix and cell surface can also promote apoptosis of SMCs leading to thinning of the fibrous cap, and influx of macrophages, thereby expanding the lipid core. As a result, proteinase expression in vulnerable
human plaques predicts adverse clinical outcomes (82). Macrophages are the most active producers of proteases in plaques and both macrophages and proteases co-localise with areas of matrix degradation. Studies in genetically modified mice and rabbits demonstrate directly the pathogenetic role of several MMPs and cathepsins in plaque instability (83).

Inhibiting proteases directly or preventing their secretion into the plaque extracellular matrix appear attractive pathways to new plaque stabilizing treatments. Gene transfer of tissue inhibitor of metalloproteinases (TIMPs), for example, increases markers of plaque stability in animal models, although non-selective chemical inhibitors of MMPs have so far been ineffective. Potent, more selective inhibitors of some MMPs are currently being evaluated in animal models. An alternative approach is suggested by recent work showing that while even naïve macrophages secrete some proteases, classical and alternative activation of macrophages increases their repertoire of proteases. M2-like, MMP-12 positive FCMs occur selectively around the lipid core of rabbit and human plaques (84). M1-like FCMs in rabbit plaques are MMP-14 positive and TIMP-3 negative; as a result they are more destructive and invasive, proliferate more rapidly and undergo apoptosis more readily, all properties expected to promote plaque instability (85). Reducing classical and alternative activation of FCMs might therefore stabilise plaques by reducing excessive protease production.

Platelets

Beyond their eminent role in haemostasis and thrombosis, platelets contribute to the formation, progression and exacerbation of atherosclerotic plaques through their secretory functions and as important modulators of inflammatory and immune responses (86, 87) but the role of platelets and coagulation in plaque stability remains to be elucidated. Most notably, plaque progression and inflammation is promoted by deposition and synergistic functions of platelet chemokines, e.g. RANTES/CCL5, on the arterial surface triggering monocyte arrest and macrophage infiltration. The disruption of hetereric interactions between these platelet chemokines by a cyclic peptide resulted in a marked reduction of athero-progression and a more stable plaque phenotype in hyperlipidaemic ApoE−/− mice (88). Likewise, clopidogrel as an anti-platelet agent widely used in cardiovascular prevention attenuated atheroma formation but increased fibrous area and stability (89). Clinically, elevated plasma levels of CCL5 specifically predict refractory symptoms and future events in unstable angina pectoris (90).

Thrombophilic conditions, such as factor V Leiden or pro-thrombin mutations have not been confirmed as clinically relevant, although elevated plasma levels of CCL5 specifically predict refractory symptoms and future events in unstable angina pectoris (90, 91). Thus, platelets and coagulation appear to have hitherto underappreciated effects on plaque stability and subsequent complications. However, experimental studies strongly support the validity of anti-inflammatory approaches to promote plaque stability.

Endothelial dysfunction and vulnerable plaque dynamics

Impairment in endothelium-dependent vasodilatation is considered to be the clinical hallmark of endothelial dysfunction, and studies on the diameter response of coronary arteries to intra-coronary infusion of acetylcholine were the first to directly translate the laboratory work by Furchgott et al. into the clinical arena (93). This type of testing has shown a heterogeneous response pattern in diseased coronary arteries, i.e. the co-existence of normal dilation and paradoxical constriction in the same vessel or the same patient. Intriguingly, compared with segments with a normal response to intracoronary acetylcholine, coronary artery sections with abnormal vasoconstriction to acetylcholine have smaller lumen area and larger plaque burden, specifically larger necrotic core areas and dense calcified plaque areas by virtual histology (VH)-intravascular ultrasound (IVUS) (94). Necrotic core area seems to be the key IVUS parameter to correlate with endothelial dysfunction, and it was calculated that for every increase of 0.01 mm² in plaque area, the relative risk of endothelial dysfunction increased by 34%.

Endothelial dysfunction is inherently associated with the atherosclerotic disease process. The dysfunctional state of the endothelium links to increased permeability of particles such as LDL and inflammation of the vascular wall (95). Based on these two processes alone, it is conceivable that dysfunctional endothelium contributes to the development of a lipid-rich and inflamed plaque. Much of these changes have been attributed to the influence of biological and physical risk factors, such as hypercholesterolaemia and shear stress, respectively, leading to an alteration in the endothelial gene expression profile. This translates into an activation of oxidative stress pathways such as NAD(P)H oxidase, the endogenous endothelin system, and most importantly, a downregulation of nitric oxide (NO) bioavailability. The latter has been considered to be the molecular hallmark of endothelial dysfunction and relates to decreased production by endothelial NO synthase (eNOS) and increased consumption (93). Superoxide anions react with NO to form peroxynitrite, which then interacts with tetrahydropterin (BH4), decreasing its availability as a co-factor for eNOS. Under these circumstances, eNOS becomes no longer the source of NO but rather superoxide anions (so-called “eNOS uncoupling”) (96). The stimulation of the oxidative stress pathway therefore leads to a self-perpetuating cycle of NO depletion. The cardiovascular consequences of this process have been well-established in experimental models of pharmacological inhibition of NO synthase, which demonstrated aggravation of atherosclerotic plaque formation (97). Conversely, intervention aimed at up-regulating the NO pathway demonstrated an attenuation of the atherosclerosis process in a predisposing environment (98).

Population-based studies identified coronary endothelial dysfunction as an independent predictor of major adverse cardiac events, and noted that preserved endothelial function attenuates the risk of future events in patients with high plaque burden (99–101). The causal aspect of this association is strengthened by the fact that acute triggers of cardiovascular events are remarkably linked to endothelial dysfunction. For instance, cardiovascular

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events follow a circadian periodicity with a peak incidence in the early morning hours. Intriguingly, patients with cardiovascular disease (CVD) who suffered from acute coronary syndrome (ACS) show a loss of diurnal variation in endothelium-dependent vasodilation that may counteract potentially adverse diurnal variations in other biological factors (102). Furthermore, in the setting of increased sympathetic activity, as it occurs in the early morning but also with other known triggers of acute cardiovascular events such as stressful physical exercise, mental and emotional stress, and cold exposure, the dysfunctional endothelium is sensitised to respond with vasoconstriction (103). These dynamics can lead to erosion, fissure and rupture of vulnerable plaque areas of reduced mechanical strength. In the presence of an activated and dysfunctional endothelium, platelet activation and aggregation is enhanced due to reduced production of NO, prostacyclin (PGI2) and likely ecto-adenosine phosphatase (ADPase)/nucleoside triphosphate diphosphohydrolase (NTPDase-1)/CD39 (104, 105). The release of peptides such as serotonin and thrombin from activated platelets leads to further potent vasoconstriction and enhances the outlined dynamics. Acute thrombotic occlusion is furthermore favoured by reduced production of tissue plasminogen activator and thrombomodulin and an increased production of tissue factor by a dysfunctional endothelium (106, 107).

Particularly in the setting of severe vasoconstriction, shear forces at the plaque can increase to such a level that it would cause marked endothelial damage followed by platelet deposition and thrombus formation (108). Detachment of endothelial cells from the collagen IV-rich basement membrane in these areas is favoured by the production of type IV collagenases (MMP-2 and –9) by activated endothelial cells and inflammatory cells (109, 110). Moreover, under the influence of granulocyte macrophage colony-stimulating factor, macrophages express and release myeloperoxidase, which can bind to the extracellular matrix and convert chloride anion and hydrogen peroxide to hypochlorous acid, thereby inducing endothelial cell death and detachment (111). Finally, low and oscillatory shear stress in the downstream part of the atherosclerotic plaque can induce endothelial cell apoptosis and detachment with mural thrombus formation by modulation of α/-β-integrin expression and caspase-3 activity (112, 113).

Adventitial, but not plaque microvessels have been detected in all animal models from mice to primates, and available data argue in favour of a relationship with atherosclerosis. First, the anatomical sites predisposed to atherosclerosis show an increased density of adventitial microvessels in mice (116) and pigs (117). In addition, adventitial and intimal blood flow measured by microspheres was substantially increased in atherosclerotic compared to non-diseased primates (118). Secondly, experimental studies support a role for adventitial microvessels in the initiation and/or progression of atherosclerosis. Even before the plaque initiation phase, adventitial microvessel density has been reported to increase in hypercholesterolaemic pigs (119). Another study suggests that adventitial microvessels only stimulate, but do not initiate, intimal thickening (120). This suggests that abundant plaque angiogenesis is not a requirement for atherogenesis, but may rather be a response to the pathophysiological state of the arterial wall. The histological detection of intra-plaque haemorrhage is associated with plaque rupture (121). However, it remains to be demonstrated, whether the intra-plaque haemorrhage from neovessels triggers plaque rupture or vice versa. Nevertheless, adventitial microvessels are clearly related to atherosclerotic disease and currently provide the only acceptable option to study (anti)angiogenic interventions in animal models of atherosclerosis.

The inhibition of angiogenesis using two independent angiogenesis inhibitors endostatin and TNP-470 resulted in the attenuation of plaque growth in apoE−/− mice (122). It has been suggested to use anti-angiogenic therapy for plaque stabilisation in humans (123). However, patients receiving anti-angiogenic therapy for cancer (e.g. VEGF-inhibitor Avastin) show a higher incidence of cardiovascular events (124). This fact is compatible with the vaso-protective effect of physiological VEGF concentrations, and short-term treatment of LDLR−/− mice with VEGF has not increased atherosclerosis (80). The final proof, however, whether the therapeutic manipulation of plaque angiogenesis can stabilise advanced human atherosclerotic plaques, is still lacking.

**Do circulating progenitor cells contribute to plaque progression?**

Subsets of mononuclear cells in the blood termed *endothelial progenitor cells* (EPCs) and *smooth muscle progenitor cells* form colonies in cell culture with endothelial or smooth muscle cell-like features, respectively (125, 126). These circulating cells, assumed to originate solely or mainly from the bone marrow, have been claimed to home and differentiate into *bona fide* endothelial and SMCs in atherosclerotic lesions in mouse models (127, 128).

Clinical studies have measured the level of these putative progenitor cells in the blood of patients and have found correlations with atherogenic risk factors, established atherosclerotic disease, and future atherosclerotic events (129–132), although contrasting directions of the correlation have also been reported, and confounding factors, such as statin treatment, may be involved (133, 134). These studies have fostered the theory that circulating pro-

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Can diet and environmental factors influence plaque stability?

Due to the multitude of dietary ingredients it is difficult to establish the ones that can have a direct or indirect effect on the stabilisation of plaques. Unfortunately, a large proportion of the data on this subject was obtained in animal studies. Nevertheless, the current meta-analysis of studies which included over 1.5 million participants suggests that greater adherence to Mediterranean diet significantly (by 9%) reduces cardiovascular mortality (142). The effect seems to be related to increased consumption of nutrients, such as folates, omega-3 acids, polyphenols and vitamin D.

Currently, we have at our disposal only one clinical study directly related to the effect of nutrients on stabilisation of the atherosclerotic plaque. It applies to the role of omega-3 acids in plaque remodelling in patients awaiting the endarterectomy procedure (143). The study included 162 patients who were administered 1.4 g/day of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (fish oil) or 3.6 g of linoleic acid (sunflower oil) for a mean time of 42 days (7 to 189 days). During the procedure, carotid plaques were removed and then their composition and structure were compared with the control. It was shown that only EPA and DHA, when incorporated into the plaque, reduced its content of macrophages (CD68 positive cells). This also resulted in an increase in the well-formed fibrous cap and a reduction of the thin inflamed cap in omega-3 treated patients. This effect was correlated with the length of administration of EPA and DHA and increased significantly after 42 days.

More experience with the action of nutrients on plaque stabilisation originates from animal experimental studies and \textit{in vitro} studies. Recently, it has been shown that lycopene administered intragastrically to rabbits on high-fat diet is equally effective as fluvastatin in the limitation of plaque formation (144). On the other hand, quercetin effectively inhibits the expression of MMP-1 in human vascular endothelial cells through blockade of the ERK pathway (145). Quercetin is also effective in the inhibition of oxidative stress due to reduction in the expression of the p47phox subunit of the NADPH-oxidase (146). Much interest is also devoted to the role of anthocyans obtained from black rice in the process of atherosclerotic plaque stabilisation in apoE-/- mice (147). It was shown that anthocyans were equally effective as simvastatin in reducing the occurrence of large necrotic core and thin fibrous cap in plaques in comparison with the control (148). Anthocyans also seem to have the ability to inhibit the production of matrix MMPs and to reduce apoptosis of endothelial cells.

Unfortunately, positive effects related to the dietary presence of biologically active nutrients, which potentially stabilise the plaques, may be reduced by substances forming as a result of thermal processing of food, e.g. trans-fatty acids, oxidised lipids and acrylamide. There are numerous data showing that they may induce chronic inflammation and increase the CRP level (149).

Hyperhomocysteinaemia

Hyperhomocysteinaemia may be caused by both genetic factors (most commonly, the C677T polymorphism in the methylenetetrahydrofolate reductase, MTHFR, gene) as well as lifestyle. It is known that insufficient supply of folic acid and B group vitamins accompanied by a high intake of methionine, as well as regular drinking of coffee and tobacco smoking, have a significant impact on the elevation of serum homocysteine levels. In addition, many drugs – including diuretics, metformin and fibrates – may result in increased levels of this amino acid. Currently, several fundamental mechanisms are indicated as being responsible for the pro-atherosclerotic activity of homocysteine. The most commonly mentioned ones are the role of homocysteine in the induction of oxidative stress, interaction with NO, formation of Sirtuinosohomocysteine and asymmetric dimethylarginine, as well as interaction with the coagulation and fibrinolysis system. The fact that homocysteine, at higher levels, stimulates von Willebrand factor, lowers protein C activity and increases thromboxane synthesis may suggest its role in inducing acute coronary events (150).

Smoking

Active and passive smoking remains one of the main risk factors for CVD (151). It should be emphasised that nearly half of the sudden deaths in males aged 40–45 is associated with smoking. The underlying causes are damage to vascular endothelium, disturbances in the coagulation and fibrinolytic system, and an intensified inflammatory reaction to toxic gasphase substances in tobacco smoke. Long-term smokers have elevated serum CRP, fibrinogen and homocysteine levels as compared to non-smokers and those who have quit smoking. Furthermore, it is known that smokers...
have elevated soluble adhesion molecules, VCAM-1 and ICAM-1, and that their monocytes exhibit stronger expression of integrins CD11b/CD18. It should be noted that, in smokers, the level of HDL fraction responsible for the reverse cholesterol transport is inversely proportional to the number of cigarettes smoked. It is known that nicotine causes an increase in serum thromboxanes A2 and B2, which reflects enhanced platelet activation. Nicotine induces an intensified platelet activity through its influence on the secretion of catecholamines.

It should be emphasised that acute coronary events occur on average 10 years earlier in smokers than in non-smokers, and carry a higher risk of Q-wave MI and sudden cardiac death (152). It has been previously shown in many studies that quitting of smoking after surviving an acute MI may result in a reduction in total mortality by as much as 36% during the 3–7 years after the event (153).

Detection of unstable plaques by imaging, biomarkers and genetic testing

The natural history of coronary atherosclerosis in individual patients is relatively unpredictable and clinical events may strike without any warning. There have been long standing efforts to discover new imaging techniques, biomarkers and genetic tests in order to diagnose patients at high risk for acute manifestations of atherosclerosis and to test and predict treatment efficacy.

Until recently it was largely unknown if assessed vulnerable plaque characteristics contain positive predictive value to identify plaques that are prone to rupture. Autopsy studies provided insights in the prevalence of histological characteristics (see above) with increased plaque vulnerability. In addition, these observational studies showed that features of plaque vulnerability are also frequently observed in asymptomatic patients, and plaques lacking typical vulnerable histopathological characteristics are able to cause clinical events. Furthermore, plaque rupture itself is also often asymptomatic (4, 11).

New approaches for risk assessment have been introduced that are based upon 1) tests on peripheral blood samples for the presence of a specific atherosclerosis biomarker; 2) genetic testing to determine propensity to unstable atherosclerosis; and 3) new imaging techniques to assess vulnerability of plaques.

Biomarkers

A good biomarker needs to be specific for disease development or progression, to have a high predictive value for events and, if possible, should reflect successful treatment. Atherosclerosis is a multi-factorial disease and therefore one single biomarker will not likely be sufficient to reach all these objectives. However, with the currently available circulating biomarkers, even the use of multiple biomarkers only adds moderate predictive value to the traditional CVD risk factors. In the Framingham heart study a cohort of 3,209 patients was analysed to evaluate if this multimarker approach could enhance risk stratification with currently available and previously reported individual biomarkers (154). A combination of 10 biomarkers was assessed and after a follow-up of 7.4 years the hazard ratio for CVD events was only 1.84 for the people in the highest quintile scores, compared to the two lowest quintiles. A large body of literature supports the idea that inflammation (and in particular CRP) plays a pivotal role in all phases of atherosclerosis, from the fatty streak lesion formation to the acute coronary event due to vulnerable plaque rupture. Indeed, vascular inflammation contributes to the pathogenesis of atherosclerosis, and later in the disease process, it is a major determinant for the acute coronary syndromes. There are various inflammatory markers that have been shown to predict cardiovascular events. These include high-sensitivity CRP (hs-CRP), a simple downstream marker of inflammation, recently emerged as a major cardiovascular risk factor (155). Elevated baseline concentrations of hs-CRP are associated with the risk of atherosclerotic events in general populations and show a predictive value even in terms of secondary prevention, both in patients with chronic stable angina and ACS (156, 157). If CRP is just a biomarker or also causally involved in plaque formation is still under debate (158, 159).

Chemokines and cytokines seem very promising targets (160), and there are also other interesting reports in this regard on e.g. tissue metalloproteinases, haemostatic factors and myeloperoxidase (161–163), but they all need still validation and confirmation. Given these results there is a pressing need for more specific and prognostic biomarkers to be added to the established risk factors to optimise risk prediction. Currently studies are ongoing that use alternative sources for biomarker discovery for the progression of atherosclerotic disease. The local atherosclerotic plaque is considered as a source for biomarkers to predict future systemic adverse events (164).

Detection of unstable plaques by genetic testing

Evidence accumulated over decades convincingly demonstrates that family history of atherosclerosis in a parent or a sibling is an important risk factor associated with CVD. The contribution of genetic factors to CVD is well illustrated by twin studies. In a study of mono- and dizygotic twins, death from CVD at an early age of one twin was a strong predictor of the risk of death of the other twin. The fact that this risk was greatest in monozygotic compared to dizygotic twins indicates a strong contribution of genes to CVD (165). The genes that contribute to this common disease are without doubt many and they code for proteins involved in multiple metabolic pathways. Such genes include those controlling lipoprotein metabolism, vascular tone and reactivity, macrophage structure and function, and haemostatic as well as fibrinolytic pathways.

In recent years, genetic testing of patients has become popular to identify individuals that are prone to clinical events by having plaque rupture of unstable plaques. First single nucleotide polymorphisms (SNPs) in candidate genes were investigated (166, 167) followed by
array analyses of multiple SNPs at the same time (168), and extensive genome wide association studies (GWAS) (169). These analyses have detected many genetic loci and SNPs which are associated with CVD. Approximately 160 genetic loci have been identified which are associated with increased or decreased risk of diseases like coronary artery disease, MI and restenosis (169, 170). Many genetic associations still lack a functional understanding and are only statistically associated with CVD. Some of these loci direct biochemical pathways which were previously not associated with CVD.

However, are these loci or SNPs currently able to successfully identify patients who have, or might develop, vulnerable atherosclerotic plaques? Various genes and proteins are seen to be involved at different stages of disease process, which could lead to TCFA. There are no data yet that clearly pinpoint to a specific genetic signature of the vulnerable plaque, but instead many genetic pathways are involved in different ways in its formation. Although a specific (single) genetic test to identify a patient who carries rupture prone plaques is the ultimate goal, this seems currently unlikely. An alternative approach is to identify the “vulnerable patient” via gene-protein signatures, which is in part mediated by the genetic background and in part by environmental factors.

Do the associated genetic traits harbor any true potential of accurately predicting future plaque destabilisation and can these traits be extrapolated to an individual patient? This is currently not yet the case, and clinical use of genetic arrays together with traditional risk factors is not yet operational. Genetic screening, however, still harbors great potential. Therapeutic stabilisation of plaques may be developed, targeting newly discovered pathways involved in plaque destabilisation. For instance, monoclonal antibodies against chemokine receptors are currently under development as some chemokines are associated via genome-wide association studies (GWAS) and protein profiling studies with CVD.

Over the past decades, it has become increasingly clear that part of the gene-environmental interactions relevant for complex diseases is regulated by epigenetic mechanisms, such as histone acetylation and DNA methylation (171). Epigenetic processes modulate gene expression patterns without modifying the actual DNA sequence and have profound effects on the cellular repertoire of expressed genes. They contribute to the expression of genes that play key roles in extracellular matrix formation, inflammation, and proliferation, processes involved in CVD. Therefore, we think it is likely that epigenetic regulators involved in histone acetylating and methylating activities contribute to the pathogenesis of atherosclerosis and restenosis. Thus, as alterations in chromatin structure are reversible, these epigenetic modifications are amendable to pharmacological intervention, which may prove to be an effective treatment modality for the management of CVD (171, 172).

Detection of unstable plaques by imaging

The ability to detect vulnerable plaques in vivo would be essential to study their natural history and to evaluate potential novel therapeutic interventions. Unstable plaques, TCFA, have an inflamed thin fibrous cap overlying a large necrotic core. Most of these pathological characteristics have been found in patients by means of non-invasive and invasive imaging modalities, which have made significant progress over the last years.

Assessment of plaque burden

In pathology, TCFA have < 50% diameter stenosis in over 75% of cases (14). By means of multidetector computer tomography, the plaque area in ruptured plaques was larger than in stable lesions (173). Using IVUS, patients with acute MI had a larger plaque area compared to patients with unstable angina and stable angina (174). This technique allows calculation of plaque volume and – to a certain extent – tissue characterisation of plaques through their echogenicity. For example, hyperechogenicity indicates dense, fi-
brous or elastic tissue and hypoechochogenicity indicates lipid or loose necrotic tissue.

VH-IVUS is an emerging variant of IVUS, using integrated backscatter signals to build an electronic colour-coded map from calculations of the radiofrequency signal values (Fig. 3). This technique allows for characterisation of calcium necrotic core, fibro-fatty, and fibrous tissue. Based on this, lesion types/plaques can be classified as fibrous, fibro-calcific, fibro-atheroma and TCFA which are considered to be at high risk for plaque rupture (175). A third technique that has been used to assess plaque stability is angioscopy, a technique that allows direct visualisation of plaques and surface irregularities (176). However, the usefulness of this method remains somewhat unclear.

Assessment of positive remodelling

Vessel remodelling can readily be evaluated with IVUS. Greyscale IVUS examination allows the identification of positive or outward vascular remodelling that is defined as a compensatory mechanism. In several IVUS studies positive vessel remodelling has been identified as one of the features associated with culprit coronary lesions and is also frequently observed in ruptured plaques (177). In unstable angina patients, outward remodelling has been defined as a significant independent predictor of major adverse cardiac events. The remodelling pattern has also been correlated with the plaque composition using VH-IVUS. A positive correlation between outward remodelling and necrotic core and a negative correlation between outward remodelling and fibrous tissue have been found (26).

Assessment of fibrous cap

While non-invasive imaging modalities and also IVUS do not have enough resolution to evaluate in detail the fibrous cap, OCT, as corroborated by histological examinations, is able to provide accurate measurements of the thickness of the fibrous cap (Fig. 3). In a clinical study combining IVUS, OCT and angioscopy in acute MI, Kubo et al., demonstrated that the incidence of TCFA was 83% and only OCT was able to estimate the fibrous cap thickness (mean 49 ± 21 μm) (26). It has been suggested that the ability of OCT to measure changes in the fibrous cap thickness could be useful to assess the effect of statins in plaque stabilisation. Furthermore, some data suggest that new OCT technology (such as polarisation-sensitive OCT) could be able to assess the collagen content and SMC density in the fibrous cap (178).

Necrotic core characterisation

Cheruvu et al. reported that the necrotic core size was different in TCFA and in ruptured plaques (16). VH-IVUS can potentially identify different plaque types including TCFA. In patients with ACS who underwent IVUS of all three epicardial coronaries, on average, there were two IVUS-derived TCFA per patient with half of them showing outward remodelling (179). In the PROSPECT trial, a multi-centre, natural history study of ACS patients, all patients underwent PCI in their culprit lesion at baseline, followed by an angiogram and VH-IVUS of the three major coronary arteries. At three-years, the highest risk plaque type being TCFA with a large plaque burden had a 17.2% likelihood of causing an event within three years (hazard ratio [HR] 10.8, p<0.001).

Thus, imaging techniques have made clear progress in identifying vulnerable plaques and have increased our understanding of pathology in vivo. Techniques emerging for future use include coronary CT, magnetic resonance imaging and imaging techniques using markers of metabolic activity of certain cell types (e.g. macrophages) such as fluorodeoxyglucose positron emission tomography (18-FDG-PET). Developing such techniques remains a challenge for the future (180).

Plaque stabilisation: Clinical trials, current treatments and future perspectives

Several therapeutic strategies have been tested for the pharmacological stabilisation of vulnerable plaques and some have entered current treatment guidelines (181). For example, the FATS study (familiar atherosclerotic treatment study, FATS) demonstrated only a very moderate reduction in angiographic stenoses in patients receiving lovastatin plus colestipol and in those randomised to niacin plus colestipol compared with those receiving standard care (182). In contrast to these angiographic results, patients assigned to the lipid-lowering strategy experienced an overall reduction of 73% in CVD events (death, MI or ischaemic events requiring intervention) compared with usual care. Similar data were found in the STAR study (St. Thomas atherosclerosis regression study) randomising patients to colestipol (183). Based on these data the hypothesis was raised, that lipid-lowering therapy may stabilise atherosclerotic lesions and decrease the likelihood of plaque rupture thus e.g. reducing the incidence of MI. With respect to therapeutic strategies to stabilise such lesions, however, current knowledge is still limited.

Statin therapy

Several statin trials have reported a 20% risk reduction in vascular events for each mM of reduction in the LDL cholesterol level in secondary prevention. Statins have been shown to stabilise atherosclerotic plaques by reducing plaque lipids and thrombogenicity,
improving endothelial function and by their antiinflammatory action (184).

To date, two studies have examined the effect of statin therapy on plaque inflammation and plaque stability. In the first study, patients received pravastatin or no treatment three months before surgery. Plaques were then removed and analysed for lipid content, inflammatory cells and collagen content. This study demonstrated that patients receiving pravastatin exhibited significantly higher collagen content and less inflammatory cells, suggesting that these plaques were more stable than plaques from untreated patients (185). The ATROCAP study (atorvastatin and thrombogenicity of the carotid atherosclerotic plaque) randomised patients eligible for two-step bilateral carotid endarterectomy to atorvastatin or placebo for 4–6 months following the first procedure. In this study, post-treatment plaques from patients treated with atorvastatin showed a trend towards fewer macrophages and inflammatory cells than pre-treated plaques, whereas no change was observed in patients receiving placebo. In addition, patients receiving atorvastatin exhibited less tissue factor content in post-treatment plaques, suggesting additional anti-thrombotic effects of atorvastatin. However, many of these changes did not reach significance, most likely due to the small sample size and a high intra- and inter-individual variability (186). Still, the results of these two studies were consistent with experimental findings that statins – in addition to their lipid-lowering properties – exhibit pleiotropic, antiinflammatory effects which are likely to contribute to plaque stabilisation in treated patients (184, 187). Such mechanisms could play a role in the large reduction of CVD events in clinical trials. Overall, immunohistochemical studies on endarterectomy specimen have contributed to our understanding of plaque stabilisation but due to the small number of patients and limitations of the technique, this approach is hard to prove a plaque stabilisation effect of a single drug.

Other studies have used IVUS to invasively characterise plaques of treated patients. The German Atorvastatin Intravascular Ultrasound Study (GAIN) demonstrated that hyperechogenicity of plaques significantly increased in statin-treated patients after 12 months compared to usual care (188). However, decreasing plaque growth and hypoechogenicity under atorvastatin did not reach significance, likely due to small sample size. Using VH-IVUS technique some trials have shown that statin therapy can increase the fibrous tissue volume, decrease the lipid core and increase the fibro-fatty plaque volume, again suggesting that statin therapy may stabilise atherosclerotic lesions (189). One small study used angioscopy to assess the effect of atorvastatin on plaque morphology in patients with CVD. It was shown that yellow and disrupted scores indicative of plaque instability significantly decreased in the statin-treated patients (176). However, this technique has major limitations because of the subjectivity and lack of clear validation.

Large trials evaluating the effects of aggressive lipid lowering with statins have shown beneficial effects on plaque size and CVD events. In the REVERSAL study, aggressive lipid lowering with atorvastatin 80 mg daily decreased hs-CRP levels and regressed the atheroma as shown with IVUS (190). Similar findings have been reported for different statins and were generated with other techniques such as MRI and angioscopy. Moreover, aggressive lipid lowering shows even a clinical benefit in primary prevention in the presence of normal cholesterol levels: The JUPITER study randomised 17,802 healthy subjects with LDL less than 3.4 mM and hs-CRP above 2 mg/l to 20 mg rosuvastatin or placebo (155). Rosuvastatin significantly reduced the incidence of major CVD events in this low-risk group.

ACS is the most urgent clinical indication for plaque stabilisation. The PROVE-IT study randomised 4,162 patients with ACS to pravastatin (40 mg daily) or atorvastatin (80 mg daily) and followed them up for 24 months (191). The incidence for the primary endpoint, consisting of death, MI, unstable angina and revascularisation, was decreased by 16% in the aggressive treatment arm. The patients deriving most benefit were those who decreased both LDL cholesterol and CRP levels. The ARMYDA-ACS study randomised 171 patients with non-ST elevation ACS to atorvastain or placebo before percutaneous coronary intervention (192). One month follow-up revealed a significant decrease in major CVD events in the statin group. These studies contribute to our understanding about the beneficial effects of statins on cardiovascular morbidity and mortality.

**Antiplatelet therapy**

Antiplatelet therapy may stabilise the vulnerable patient by reducing the amount of local thrombus formation as well as reducing vascular inflammation (193). Aspirin has been shown to be effective for secondary prevention in patients with established atherosclerotic vascular disease. Out of the four commonly recommended therapies for secondary prevention (statin, aspirin, beta blocker, angiotensin-converting enzyme inhibitor), the combination of statin and aspirin are associated with the greatest reduction in mortality in a case-control analysis. In addition to aspirin, there is also positive evidence for other antiplatelet agents such as clopidogrel, prasugrel and ticagrelor. The CURE trial showed a major reduction in CVD events when clopidogrel was added to aspirin in patients with ACS (193). The positive effects of prasugrel (TRITON) (194) and ticagrelor (PLATO) (195) on CVD events were even greater.

**Anti-hypertensive therapy**

Beta blockers have been shown to reduce recurrent MI, sudden cardiac death and total mortality in patients with MI in several clinical trials (196). They reduce heart rate and blood velocity resulting in less turbulent flow and lower wall stress. A recent pooled analysis of four IVUS trials has shown that beta blockers slow the progression of atherosclerosis (197).

Angiotensin II is a proinflammatory cytokine and augments the production of reactive oxygen species. Blocking angiotensin II has been shown to reduce signs of inflammation in atherosclerotic ani-
Other anti-atherosclerotic therapies

The therapeutic option of lowering LDL and VLDL cholesterol while raising HDL cholesterol using nicotinic acid has recently received new emphasis, given the strong inverse relationship between CVD risk and HDL cholesterol (201). Niacin decreases CVD and the progression of atherosclerosis. The identification of a G-protein-coupled receptor for nicotinic acid may yield insights into how this compound leads to a favourable alteration in HDL cholesterol, and to pleiotropic anti-inflammatory effects, and may provide a platform for developing candidate molecules without side effects. Recently, two clinical trials indeed revealed that in statin-treated patients with low HDL-C, high-dose modified-release nicotinic acid, compared with placebo, significantly reduces carotid atherosclerosis within 12 months (202), and that the use of extended-release niacin caused a significant reduction of carotid intima-media thickness when combined with a statin, demonstrating that niacin is superior to ezetimibe (203).

Artificial HDL-like apoA1 complexes, especially apoA1-Milano have been shown to reduce atherosclerotic plaques (204). Recently, the large GISSI-P trial has shown omega-3 supplementation to have an additional effect on reducing CVD events (205). Likewise, PPAR agonists have shown plaque stabilising effects in several studies although the clinical evidence is conflicting (206). The effects of anti-diabetic thiazolidindiones on plaque stability have been studied. In one study, non-diabetic patients were randomised to placebo or rosiglitazone for six weeks prior to carotid endarterectomy, and after surgery plaques were analysed. Rosiglitazone significantly reduced CD4-positive lymphocyte content without having an effect on the number of plaque macrophages. However, macrophage activation, as assessed by HLA-DR staining, was significantly reduced which resulted in an increase in collagen type-I content, suggesting that rosiglitazone treatment may convert unstable plaques to more stable plaques. Interestingly, the observed results were independent of the glucose-lowering and lipid-modifying properties of this drug (207). However, large clinical trials, for example the RECORD trial failed to show a reduction in CVD events in rosiglitazone-treated patients with type 2 diabetes, questioning the overall benefit of such plaque stabilising effects of rosiglitazone (208).

The use of selective cannabinoid type 1 receptor antagonist rimonabant in patients with abdominal obesity and metabolic syndrome failed to achieve a favourable effect on the progression of CVD, as measured by % atheroma volume (209). Likewise, the cholesteryl ester transport protein (CETP) inhibitor torcetrapib, which increases HDL-cholesterol and decreases LDL-cholesterol, failed to decrease progression of coronary atherosclerosis at lower dose but, induced regression at the highest HDL-cholesterol levels (210). However, at the highest dosage, torcetrapib raised serum sodium and lowered potassium, consistent with an aldosterone-like effect. This may explain the lack of benefit in the full study cohort but also implies that CETP inhibitors devoid of this off-target toxicity may still have the potential to halt plaque progression.

A new approach to treat plaque inflammation is by targeting the activity of lipoprotein-associated phospholipase A$_2$ (Lp-PLA$_2$), which primarily acts on Ox-LDL (211). Ox-LDL in vulnerable plaques is known to generate pro-atherogenic compounds, such as lysophosphatidylcholine and is associated with increased risk of CVD events. Indeed, selective inhibition of Lp-PLA$_2$ with darapladib reduced the development of advanced coronary atherosclerosis in diabetic and hypercholesterolaemic swine, decreasing lysophosphatidylcholine content, necrotic core area and frequency of lesions with an unstable phenotype (212). Darapladib exerted anti-inflammatory effects, reducing the expression of macrophage- and T-cell function-associated genes, such as CCL5 and cathepsin S. In patients receiving high standard-of-care treatment, the effect of darapladib on coronary atherosclerosis was compared with placebo. While necrotic core size as a secondary endpoint and key determinant of plaque vulnerability continued to expand in patients receiving placebo, this was prevented by Lp-PLA$_2$ inhibition with darapladib (213). As a co-primary endpoint, changes in number and area of regions with high strain did not differ in the overall study but showed a significant reduction in darapladib-treated patients. In the absence of safety concerns, darapladib may be a valuable option for future studies to determine favourable effects on CVD.

New approaches

Recent studies have identified strong expression of various MMPs in atherosclerotic lesions and their contribution to fibrous cap thinning by degrading extracellular matrix. This process is controlled by transcription, enzyme processing, enzyme activation, and specific inhibition by TIMPs. For instance, lesional over-expression of MMP-9 induces acute plaque disruption and promotes intraplaque haemorrhage in advanced lesions of ApoE$^{-/-}$ mice (214). Thus, the development of drugs specifically targeting lesional MMPs, namely MMP-9, may be a valuable therapeutic modality for preventing plaque progression and stabilising rupture-prone plaques.

The potent elastase cathepsin S, which co-localises with sites of elastin degradation in human coronary plaques, has also been implicated in primary atherosclerosis, as evident by reduced plaque size, intimal macrophage and lipid content in cathepsin S-deficient LDLR$^{-/-}$ mice (215). Moreover, plaques in brachiocephalic arteries of fat-fed cathepsin S-deficient ApoE$^{-/-}$ mice showed fewer ruptures and a more stable plaque phenotype (215). Using a biotinylated form of its endogenous inhibitor cystatin, active cathepsin S was detected in plaques, especially in macrophages of the shoulder regions. Beyond revealing a role of cathepsin S in plaque
Key messages
- **Concept:** Concept of plaque stabilisation emerged about 20 years ago in an attempt to explain the discrepancy between the reduction of cardiovascular events in patients receiving lipid lowering therapy without concomitant regression of coronary atherosclerosis in angiography.
- **Vulnerable plaque:** Vulnerable plaque is prone to rupture and thrombosis. There are two major types of vulnerable plaques, rupture-prone and erosion-prone. The prototype of a rupture-prone plaque contains a large and soft lipid-rich necrotic core covered by a thin and inflamed fibrous cap.
- **Thin-cap fibroatheroma (TCFA):** If the fibrous cap is thin, the plaque is called a TCFA. Thin fibrous caps are usually heavily inflamed.
- **Plaque stabilisation:** A plaque can be stabilised by increasing the thickness of fibrous cap, reducing inflammation in the fibrous cap and reducing the size of atheromatous core. A plaque may be stabilised against thrombosis independent of changes in plaque size and luminal obstruction.
- **Statins:** Can reduce clinical risk of rupture.
- **Imaging:** Still problematic in predicting the presence of vulnerable lesions.

Formation, destabilisation and rupture, these studies open an interesting option for therapeutic targeting with cystatins.

Direct thrombin inhibitor melagatran can reduce lesion size and promote fibrous caps and plaque stability in ApoE-/- mice, reducing activation of proinflammatory transcription factors and MMP-9 synthesis (216). Antagonists against the powerful proatherogenic chemokine receptor CCR5, which also affects MMP-9 expression, have been developed (217) and, notably, Maraviroc has been approved as an human immunodeficiency virus (HIV) entry inhibitor to reduce viral load. Hence, it would be warranted to evaluate the effects of Maraviroc on plaque progression and stability in patients with coronary artery disease, e.g. in HIV-infected patients, who are susceptible to atherosclerosis (218). As this drug has now successfully completed two phase III clinical trials in HIV-1 patients, the issue of safety has sufficiently been addressed to provide reasonably solid grounds for this endeavor.

A highly selective approach to suppress atherogenic and destabilising functions of the CCR5 ligand CCL5 without clinically relevant side effects has been recently introduced by the disruption of synergistic heteromer formation of the platelet-derived chemokines CCL5 and its interaction partner CXCL4 using a cyclic peptide approach (88). The treatment with the peptide resulted in a marked inhibition of atheroprosession and inflammatory cell content and also appears to exert beneficial effects in myocardial ischaemia-reperfusion. This compound has undergone extensive toxicological testing and is currently under clinical testing for CVD.

The chemokine-like cytokine MIF has been implicated in atheroprosession and the formation of unstable plaques with MMP-9 expression in mouse and man (72, 73). Hence, an inhibition of MIF with biologicals, e.g. peptides, or with small-molecule antagonists targeting its CXCR2 chemokine receptor agonism, as currently developed, may be similarly suited to mediate regression of atherosclerosis and stabilisation of advanced plaques, as seen with a monoclonal antibody in mouse models (74). Obviously, the validation of its effectiveness, as for other drugs and candidates, will require vigorous testing and appropriate clinical study settings, e.g. coronary atheroma analysis by IVUS.

It is well accepted that adaptive immunity regulates the extent of proatherogenic inflammation and that T-cells can affect the stability of atherosclerotic lesions. Immunisation of hyperlipidaemic animals with LDL preparations or fragments of apoB-100 reduces atherosclerosis, suggesting that vaccination may represent a useful strategy for disease prevention or modulation (219, 220). Studies applying immunisation strategies with subcutaneous injections of oxidized LDL (oxLDL) or native LDL yielded a reduction in atherosclerosis even independently of antibody titers to oxidative neoepitopes. It remains to be seen whether oxLDL is the most appropriate antigen for vaccination. Alternative options include aldehyde-modified apoB-100 peptide antigens, oxidised phospholipid antigens, heat shock proteins or other antigens, such as VEGF-receptor 2. It has been shown that immune responses elicited by transferred antigen-loaded dendritic cells directed towards antigens presented by arterial SMCs aggravate atherosclerosis (221). Presenting antigens, such as LDL, oxLDL or derived peptides in the vascular wall, dendritic cells may instigate or sustain inflammatory T-cell responses driving atherosclerosis. Notably, dendritic cell-based vaccination strategies have been successful in other contexts, such as protecting mice from autoimmunity (222). This strategy may also apply to the treatment of atherosclerosis and CVD.

In summary, for plaque stabilisation there is strong clinical evidence for statins, and also positive results for aspirin and other antiplatelet agents, beta blockers and renin-angiotensin-aldesteron system inhibitors. Also, there is some clinical evidence for PPAR agonists, niacin, omega-3 fatty acids and some HDL raising therapies for plaque stabilisation. It can be anticipated that compounds such as CETP inhibitors will be further refined, and others, such as PLAA inhibitors will be tested in larger scale trials. New targets and strategies, such as vaccination emerge to be validated, while established drugs such as niacin, owing to new formulations and consolidated insights, will receive new attention.

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Conflict of interest
None declared.
Abbreviations

ACE, angiotensin converting enzyme; ACS, acute coronary syndrome; CETP, cholesteryl ester transport protein; CRP, C-reactive protein; CVD, cardiovascular disease; eNOS, endothelial nitric oxide synthase; EPCs, endothelial progenitor cells; FCM, foam-cell macrophage; GWAS, genome wide association studies; HDL, high-density lipoprotein; HIF, hypoxia inducible factor; IFN-γ, interferon-γ; IL, interleukin; IL-4, −10, −13, interleukin-4, −10, −13; IVUS, intravascular ultrasound; LDL, low-density lipoprotein; LDR, LDL receptor; MCP-1, monocyte chemotactic protein-1; MHC-I/II, main histocompatibility complex I/II; MI, myocardial infarction; MIF, macrophage migration inhibitory factor; MMP, matrix metalloproteinase; NO, nitric oxide; OCT, optical coherence tomography; OX-LDL, oxidised LDL; PCI, percutaneous coronary intervention; PPAR, peroxisome proliferator activated receptor; SMC, smooth muscle cells; SNP, single nucleotide polymorphism; TCFA, thin-cap fibroatheroma; TFG-B, transforming growth factor-B; TIMP, tissue inhibitor of metalloproteinase; tPA, tissue plasminogen activator; VEGF, vascular endothelial growth factor; VHLD, very low-density lipoprotein; WHHL, Watanabe Heritable Hyperlipidemic rabbit.

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