

Current Advances in Understanding the Pathogenesis of Atherosclerosis and its Clinical Implications in Coronary Artery Disease.

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Abstract: Atherosclerosis is a chronic, progressive, multifactorial disease of medium and large sized arteries characterized by intimal lesions called atheroma or atheromatous plaques that protrude into the vessel lumens. Coronary artery atherosclerosis is the principal cause of coronary artery disease (CAD). It is very important to understand the fundamental mechanisms of atherogenesis in order to comprehend the adverse clinical outcomes of CAD and their subsequent management. The development of atheroma involves a number of cellular and molecular events including endothelial dysfunction, leucocyte recruitment and diapedesis, LDL transcytosis, LDL oxidation, recruitment and proliferation of smooth muscle cells, synthesis of extracellular matrix proteins, accumulation of foamy macrophages along with interplay of various cytokines. With the current advances in understanding the pathogenesis of plaque, it has been established that it is the qualitative aspect of plaques rather than the degree of occlusion by the plaques, which determine their propensity to cause acute complications. Non stenotic or vulnerable plaques are more prone to physical disruption and thrombosis. Both stenotic or stable plaque and non stenotic or vulnerable plaque can be visualized by morphologic and molecular imaging techniques. In conclusion, studying the pathophysiology of atheromatous plaque is of great importance in understanding CAD, its clinical aspects, established and emerging techniques for imaging atheroma and its role in guiding the treatment modality.

INTRODUCTION

Atherosclerosis is a progressive disease of medium and large sized arteries characterized by focal intimal lesions called atheromas or atherosclerotic plaques that protrude into vessel lumen and eventually leading to various complications¹. 'Athero' in Greek means gruel and 'sklerose' means hard. Coronary arteries are particularly susceptible to atheromas. In developed countries atherosclerosis causes more than half of total mortality, Coronary Artery Disease(CAD) is responsible for a major proportion of these deaths². The most dreaded sequel of atherosclerosis, such as myocardial infarction and stroke, are caused by superimposed thrombosis in a ruptured plaque. Therefore, the intriguing question is not why atheroma develops but rather why atherosclerosis, after years of indolent growth, suddenly becomes complicated with luminal thrombosis. If such vulnerable plaques, which are prone to rupture could be detected beforehand, it would contribute towards decreasing the morbidity and mortality due to atherosclerosis. In this respect, current advances in understanding the pathogenesis of atherosclerosis has given some major targets for the functional imaging of atheromatous plaques. Once these rupture prone plaques are detected their critical assessment and observation by the physician can avert their risk of disruption and subsequent death causing potential. Besides this, the type of plaque also guides the therapy that will benefit the patient in alleviating his symptoms.

RISK FACTORS IN ATHEROSCLEROSIS

Atherosclerosis is more likely to affect an individual if his body nests certain risk factors. Various prospective epidemiological trials have shown that the risk of developing the manifestations of coronary atherosclerosis is increased by smoking, hyperlipidemia, hypertension and diabetes². These risk factors can be prevented by lifestyle modification measures. The non modifiable risk factors are increased age, male gender and genetics and most importantly a positive family history. Still there are 20% of cardiovascular events that take place in the absence of the above mentioned risk factors. Few other factors have been implicated to explain it like increased levels of C Reactive Protein(CRP), an acute phase reactant synthesized primarily by liver, hyperhomocysteinemia, metabolic syndrome, increased lipoprotein A levels, elevated plasminogen activator inhibitor 1 levels. Last but not the least, competitive and stressful lifestyle, better known as Type A personality, is another very important risk factor for developing atherosclerosis¹.

ATHEROPROTECTIVE FACTORS

Exercise and high-density lipoprotein (HDL) and its major apolipoprotein, apoA-I play a protective role. Although, the best-known antiatherogenic function of HDL is its ability to promote the efflux of cholesterol from cells besides its antioxidant and antithrombotic properties, recent studies has uncovered the ability of HDL to paradoxically enhance vascular inflammation, lipid oxidation, plaque growth, and thrombosis due to HDL induced changes in specific enzyme and protein components³.

PATHOGENESIS OF ATHEROSCLEROSIS: "ATHEROGENESIS"

Atherosclerosis is a chronic, immunoinflammatory, fibroproliferative disease of medium and large sized arteries. The concepts of atherogenesis have evolved from vague ideas of inevitable degeneration to a well defined scenario of molecular and cellular events. It is now known that endothelial cells, leukocytes, and intimal smooth muscle cells play the key roles in the development of this disease^{4,7}. There are three stages in the life history of an atheroma i.e. initiation, progression and complication⁷.

INITIATION

Recruitment of mononuclear leucocytes to the intima characterizes initiation of the atherosclerotic lesion. Specific adhesion molecules expressed on the surface of vascular endothelial cells, under the effect of atherogenic stimuli, mediate leucocyte adhesion of mainly monocytes and to a lesser extent, T-lymphocytes to the intima. These adhesion molecules are selectins and members of the immunoglobulin superfamily such as Vascular Cell Adhesion Molecule-1 (VCAM-1), Intercellular Adhesion Molecule-1(ICAM-1). Once adherent, the leucocytes enter the intima directed by chemoattractant chemokines such as macrophage chemoattractant protein-1 (MCP-1), Tumour Necrosis Factor(TNF)⁴. Cytokines (e.g., interleukin-8) also may play a role in monocyte-macrophage trafficking. The monocytes get transformed to macrophages in the intima. With chronic hyperlipidemia, lipoproteins accumulate inside the intima, subsequently oxidized by the action of oxygen free radicals generated by intimal macrophages. Oxidized LDL further stimulates the release of cytokines and chemokines and flares up the ongoing inflammation. Macrophages internalize oxidized LDL through scavenger receptors and are then called foam cells. Accumulation of foam cells is the hallmark of early and asymptomatic atheromatous precursor, the fatty streak.

PROGRESSION

As the disease progresses, the inflammatory response is accompanied by a fibroproliferative response mediated by intimal smooth muscle cells. Progressing atheroma involves accumulation of smooth muscle cells which elaborate extracellular matrix macromolecules. Smooth muscle cells and the collagen rich matrix they produce, confer stability to plaques, protecting them against the dreaded consequences of plaque rupture and thrombosis. The smooth muscle cell is the principal connective tissue producing cell in the normal and atherosclerotic intima⁸. Synthetic activity of smooth muscle cells is regarded beneficial whereas their loss is detrimental for the plaque stability. Lack of smooth muscle cells at sites of rupture is attributed to apoptotic cell death^{9,10}. Progressing atheroma often accumulates calcium. Calcification probably has a stabilizing effect on the plaque. Neovascularisation is frequent in advanced atherosclerosis (figure 3). It is probably a marker of ongoing disease activity and characterize high-risk plaques as the

new vessels aggravate risk of intraplaque haemorrhage as well as play role in drawing in more inflammatory cells into the plaque¹¹.

An atheromatous plaque is composed of cellular component in form of macrophages, smooth muscle cells, T cells, extracellular matrix including collagen, elastic fibres and proteoglycans and lipids, intracellular as well as extracellular¹. Morphologically, there is an outer fibrous cap composed of collagen and smooth muscle cells. Deep to the cap is a more cellular area containing macrophages, T cells and smooth muscle cells. Beneath it, is a necrotic core containing lipid, debris of dead cells, foam cells, fibrin, smooth muscle cells.

COMPLICATIONS ASSOCIATED WITH PLAQUES

A stenotic plaque (figure 2) can gradually occlude a vessel, compromise blood flow and cause ischaemic injury to myocardium depending on the blood supply by the affected vessel. In the coronary circulation this occurs when there is loss of 70% of area through which blood can flow. This is known as critical stenosis (figure 1). The clinical condition is known as stable angina.

On the other hand, vulnerable plaques are more prone to acute plaque change like rupture/fissuring, erosion/ulceration and haemorrhage into the atheroma or intraplaque rupture. Intraplaque haematoma further increases the intraplaque pressure making it prone to physical disruption. Plaque vulnerability and destabilization is of multifactorial etiology with inflammation, cap matrix and necrotic lipid core remodelling being important pathobiological processes associated with vulnerability and destabilization¹². Reduced matrix synthesis as well as increased matrix degradation predisposes vulnerable plaques to rupture in response to extrinsic mechanical or hemodynamic stresses. Other contributing factors towards disruption of plaque are vasospasm, low flow, decreased fibrinolytic activity, procoagulant states etc. Modification of endothelial dysfunction and reduction of vulnerability to plaque rupture and thrombosis may lead to plaque stabilization¹³. A ruptured plaque may manifest clinically as unstable angina, myocardial infarction or sudden death. However, if the plaque disruption is minor, local flow is high, and the fibrinolytic system is active, thrombus formation may be minimal and plaque may remain silent for years and may pose life threatening sudden complications if left undetected. A thrombus may propagate and accumulate additional platelets. Older thrombi may become organized and recanalized. Recent thrombi may dissolve by fibrinolysis.

BIOCHEMICAL BASIS OF ATHEROSCLEROSIS

The thickening of artery walls is associated with deposits of cholesterol which originate from LDL particles that circulate in the blood. Current research suggest that damage to the endothelial cell inner lining of the vessel allows LDL particles and blood platelets to enter the arterial wall. The LDL contains polyunsaturated fatty acids, which can be attacked by free radicals, generated as a part of normal metabolic processes. As a result LDL becomes oxidized and gives rise to products

that are toxic to the cells of the artery wall. Macrophages attempt to remove the oxidized LDL but are unable to degrade the cholesterol, which accumulates as droplets thereby giving a foamy appearance. Cholesterol laden foam cells give characteristic appearance to the fatty streaks. The toxic products can kill the foam cells leaving cholesterol deposits, and the accumulated cholesterol, cells and debris constitute an atheroma. Conditions such as hypercholesterolemia, which is associated with defective LDL receptor increases the risk of atherosclerosis. Besides LDL there are other key players having an important role in atherogenesis as well as its prevention¹⁴. Numerous large-scale epidemiological studies persistently demonstrated an inverse relationship between plasma high-density lipoprotein cholesterol (HDL-C) level and the risk of coronary heart disease (CHD).

TYPES OF PLAQUE

On the basis of pathological characteristics, two types of plaques are identified. The first one is a vulnerable plaque, also known as non stenotic plaque which is prone to rupture, thrombosis and other complications of plaque. It has a large lipid core (figure 4), thin fibrous cap, clusters of inflammatory cells mainly foamy macrophages, fewer smooth muscle cells. Vulnerable plaque also shows outward remodelling of media, which preserves the vessel lumen known as compensatory enlargement or positive remodelling often leading to underestimation of size by X ray angiography.

The other type is a stable plaque or stenotic plaque, which is characterized by a thick fibrous cap, small lipid core, more of collagen synthesizing smooth muscle cells, less inflammation. It shows less compensatory enlargement¹². It gradually occludes the vessel well visualized by traditional angiographic techniques. Stenotic lesions give rise to blood vessel luminal compromise. These lesions are easily assessed with conventional radiographic methods.

IMMUNOHISTOCHEMISTRY IN PLAQUE

The human monoclonal antibody F16 stains areas of active tissue remodelling in atherosclerotic plaques and may serve as a tool for plaque imaging. In various studies CRP was frequently identified by IHCS in the inflammatory cells and atherosclerotic tissue of patients with unstable angina suggesting that CRP directly mediates an inflammatory process in the atherosclerotic plaque¹⁵. Annexin V immunostaining can be used as a marker of apoptosis. Smooth muscle actin and CD68 immunostaining highlights the macrophages in the plaque. In one study fibrinogen/fibrin I, fibrin II, and fibrinogen degradation products were detected in areas of loose connective tissue, in thrombus, and around cholesterol crystals in fibrous and advanced plaques implying that increased fibrin formation and degradation may be associated with progression of atherosclerotic disease¹⁶.

ASSESSING THE PLAQUE MORPHOLOGY BY MORPHOMETRY

Morphometry is done on histological sections of coronary arteries to identify the

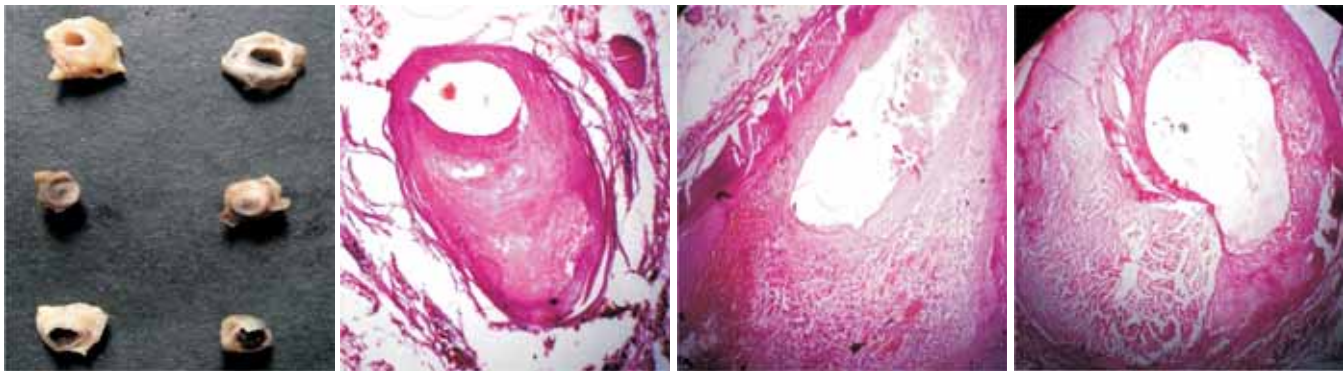


Figure 1: Gross view of coronary arteries with patent (top), stenotic plaque narrowed (middle) and thrombosed lumen (bottom).

Figure 2: H & E stained section of coronary artery with stenotic plaque (4*10X magnification).

Figure 3: H & E stained section of coronary artery with vulnerable plaque showing neovascularization (4*10X magnification).

Figure 4: H & E stained section of vulnerable plaque in coronary artery with numerous cholesterol clefts (4*10X magnification).

degree of stenosis, area of the plaque, lumen diameter, wall thickness etc. techniques employed being stereological point counting, computer assisted planimetry¹⁷.

CLINICAL IMPLICATIONS OF ATHEROMATOUS PLAQUES

From a clinical standpoint, a spectrum of acute coronary events may follow atherosclerotic plaque rupture. These events range from the asymptomatic to those resulting in critical illness or sudden death. The pathophysiology underlying these clinical events involves a reduction in blood flow supporting myocardium distal to the site of acute plaque rupture. Blood flow is reduced by accumulated thrombus, as well as vasospasm over the ruptured plaque. The severity of the resulting coronary event appears to be related to the change in blood flow around the site of plaque disruption. In those cases where blood flow is essentially unaffected, plaque rupture may result only in asymptomatic progression of the atherosclerotic lesion. If blood flow is reduced, a change in the pattern of angina may result, producing unstable angina. If complete vessel occlusion follows plaque rupture acutely in the absence of sufficient collateral blood flow, acute MI results^{18,19}. The risk of adverse outcomes after acute coronary syndromes appears to be related to the type of event. Cumulative 6-month mortality is highest in acute MI when compared with unstable and stable angina²⁰. Clinical outcome data, including cumulative death or MI and cardiac event rates, are similar for non-Q and Q-wave MI^{21,22}. The risk of adverse outcome with unstable angina is highest in the post-MI setting, or with recent (<48 h) onset of rest angina. The process of plaque rupture may play a major role in the pathogenesis of these entities. On the other hand, if external factors which trigger worsened angina (e.g., anemia, environmental stresses) can be identified and corrected, prognosis may be improved²³. Infect the treatment modality for acute coronary syndrome also depends on pathology of plaque. Although, thrombolytic therapy represents a substantial advance in the reduction of mortality after acute MI^{24,25}, but thrombolysis has not proven effective in the treatment of unstable angina and non Q wave MI²⁶. Unstable angina is felt to depend mainly on platelet-mediated mechanisms of thrombosis²⁷. As a result, unstable angina is less responsive to fibrinolytic therapies than to antiplatelet strategies such as aspirin and the glycoprotein IIb/IIIa antagonists. In fact, aspirin has been shown to improve survival and reduce recurrent vascular events in unstable angina²⁸.

IMPORTANCE OF IMAGING OF PLAQUES

It is now established that rupture of vulnerable plaques is the main cause of acute coronary syndromes most importantly myocardial infarction. Identification of vulnerable plaques is therefore essential to enable the development of treatment modalities to stabilize such plaques and prevent their progression. Several diagnostic methods are currently in use and under trial to detect vulnerable plaques.

X ray based angiography is the current gold standard for diagnosing coronary artery stenoses or the morphological imaging of the plaques. Though it does provide information about the entire coronary tree and serves as guide for invasive imaging techniques and therapy but it has a low discriminatory power to identify the vulnerable plaque. CT Angiography is now emerging as a popular non invasive technique for screening & diagnosis of plaques by imaging. It is done as an out patient procedure. Besides assessing degree of stenosis, it can characterize the plaque as fatty, fibrofatty or vulnerable and also assesses the amount of calcification in the plaque. Other less well established technique for imaging plaques is intravascular ultrasound (IVUS), an invasive technique that localizes plaques and quantifies plaque burden. Virtual Histology-Intravascular Ultrasound (VH-IVUS) can identify plaque components. Optical Coherence Tomography (OCT), also known as Optical Frequency Domain Imaging (OFDI) identifies intimal hyperplasia, also detects and quantifies the key features of vulnerable plaques.

Thus it can be said that the imaging of atherosclerosis is reaching beyond anatomy to encompass the assessment of aspects of plaque biology related to the pathogenesis and complication of the disease. Many components of ongoing inflammatory process in the plaque have been approved as imaging targets in various experimental studies of atherosclerosis. Recent biologic insights into atherosclerosis suggest imaging targets in addition to inflammatory processes. Metabolic activity of cells within atherosclerotic plaque is utilized to image plaques by means of Positron Emission Tomography (PET) imaging using 18Fluorodeoxyglucose (FDG) which is readily taken up by the foamy macrophages in the plaque. Various imaging targets being proposed and approved by pilot studies are endothelial activation markers like adhesion molecules, phosphatidyl serine which targets apoptosis within an atherosclerotic plaque, scavenger receptors on foamy macrophages,

matrix-metalloproteinases like gelatinases and collagenases, angiogenesis markers like integrin α V β 3. New concept of targeted drug delivery using specific drugs conjugated to surface of imaging particles incorporated into its structure is currently under research²⁹.

TREATMENT

Recent advances in the learning of atherosclerosis has brought forward the fact that qualitative aspects of the plaques are the chief determinants of the acute complications likely to occur in an atherosclerotic plaque. In the past era flow-limiting arterial stenoses and functional indexes of end-organ ischemia were used as a guide to therapies. But now focus is on alleviating the vulnerability of the plaque so as to prevent its complications. Beyond the lifestyle measures of exercise and diet, pharmacotherapy with statins, PPAR- α activators, and ACE inhibitors may owe their clinical benefits in part to an anti-inflammatory action³⁰.

CONCLUSION

Coronary Artery Disease (CAD) is the most frequent cause of hospitalization in western countries. The stenotic/stable plaques are less prone to disruption and rarely causes MI. They produce ischaemic symptoms which are managed by revascularization procedures. The non-stenotic/vulnerable plaques are prone to rupture and responsible for most cases of MI. Thus, it is important to identify both stenotic and non-stenotic plaques by imaging techniques. There is an urgent need to bring the identified molecular imaging targets into clinical practice. A vulnerable plaque once detected, demands critical observation by the physician so that with aggressive medical management, the chances of undergoing an invasive procedure can be lessened.

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