DIAL⊛GUES

# Atheroma

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# The birth, growth, and consequences of the atherosclerotic plaque

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*As atherosclerotic plaques develop, so does the risk of thrombosis, particularly when plaques have a high macrophage inflammatory content, a large core of extracellular lipid, a thin cap, and a reduced smooth muscle content. As the proportion of plaques with these characteristics varies widely, it is important to identify subjects with large numbers of vulnerable plaques to target aggressive therapy to those most at risk. The initial inflammatory stimulus to plaque formation is modification and oxidation of low-density lipid having crossed into the intima. This causes monocyte migration into the intima followed by lipid uptake by macrophages to form foam cells, which then release their contents after death by apoptosis and necrosis to form the plaque core. Thrombus follows either endothelial erosion or plaque disruption—both processes are mediated by release of proteases and other mediators for macrophages, such as monocyte chemoattractant protein–1 (MCP-1), whose importance is being increasingly recognized. Lipid lowering by statins has significantly reduced the risk of acute events. Animal models confirm that lipid lowering will reduce the plaque inflammatory content and initiate collagen synthesis to form a stable plaque. Lipid lowering does not totally abolish risk—there are other nonlipid stimuli of plaque inflammation that may operate as enhancers,* Chlamydia *being an example.*

**Keywords:** atherosclerosis; thrombosis; plaque formation; acute coronary syndrome; coronary disease progression; stable angina; risk factor; inflammation

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Atherosclerotic plaques are focal intimal lesions found in the aorta, iliac, femoral, carotid, coronary, and cerebral vessels. Pathologists can observe the intimal surface of arteries directly after opening the vessel longitudinally. While such studies seem artificial to clinicians, they have permitted valuable observations about atherosclerosis to be made. Plaques in adults range from yellow lines or dots barely raised above the surface (fatty streaks) to elevated dome-shaped lesions (raised fibrolipid plaques) up to 2 centimeters in their long axis in the aorta. The raised fibrolipid plaque is the basis on which clinical disease develops, largely due to thrombosis developing as a plaque complication.

| SELECTED ABBREVIATIONS AND ACRONYMS | |
|---|---|
| **ACS** | acute coronary syndromes |
| **bFGF** | basic fibroblast growth factor |
| **CRP** | C-reactive protein |
| **HSP** | heat shock proteins |
| **IL-1** | interleukin-1 |
| **MCP-1** | monocyte chemoattractant protein–1 |
| **MHC** | major histocompatibility complex |
| **MMP** | matrix metalloproteinase |
| **MSCF** | macrophage colony–stimulating factor |
| **NO** | nitric oxide |
| **PCNA** | proliferating cell nuclear antigen |
| **PDGF** | platelet-derived growth factor |
| **TGFβ** | transforming growth factor β |
| **TIMPs** | tissue inhibitors of metalloproteinases |
| **TNF-α** | tumor necrosis factor α |
| **VCAM-1** | vascular cell adhesion molecule–1 |

## THE PLAQUE BURDEN CONCEPT

When measurements of the proportion of the intima occupied by plaques are carried out in large numbers of subjects at autopsy from different geographic populations interesting messages emerge:

- The extent of intima covered by raised plaques predicts the level of clinically expressed disease in that population, ie, the plaque burden (number of plaques present) is a measure of the risk of clinical disease.

- Hypertension, diabetes, hyperlipidemia, and those who smoke have more plaques than those subjects without these risk factors.[1-3] This means risk factors operate in part by increasing the total number of plaques.

- In all populations, the extent of aortic, carotid, and coronary disease is closely related.

However, these epidemiological data apply to large patient groups rather than individuals.

## STRUCTURE OF THE RAISED FIBROLIPID PLAQUE

Plaques have many components. The connective tissue matrix proteins (collagen, elastin, and proteoglycans) are synthesized by smooth muscle cells to form the skeleton of the plaque. A variable amount of lipid, including cholesterol esters, crystalline cholesterol,
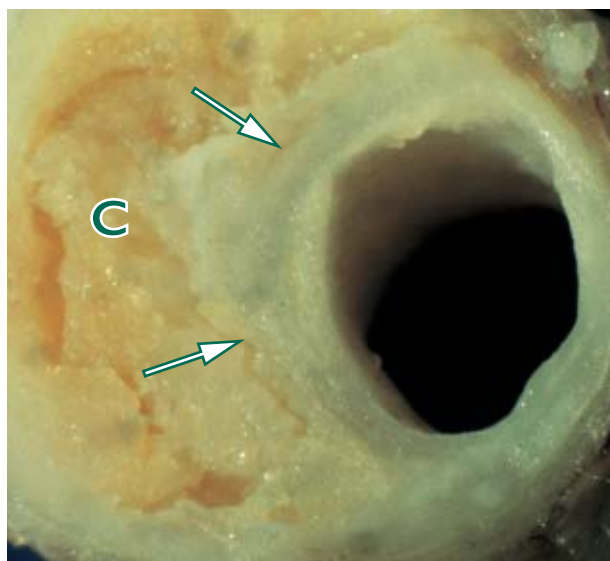


**Figure 1.** *A human coronary plaque seen in cross-section. The plaque has a core of lipid (C) separated from the lumen of the artery by a fibrous cap (arrows).*

and phospholipid, occurs both free in the tissue and within the cytoplasm of foam cells. The cellular component includes smooth muscle cells, macrophages, which form most of the foam cells, T lymphocytes, and mast cells. The adventitia contains B lymphocytes and plasma cells. The archetypal raised plaque (American Heart Association [AHA] Type IV, Va)[4] has a core of extracellular lipid contained within a capsule of collagenous fibrous tissue *(Figure 1).* The segment of the capsule separating the core from the lumen is the plaque cap. The core lipid is soft with a consistency like toothpaste. The lipid core is surrounded by large numbers of lipid-filled macrophages, a high proportion of which are activated, producing tissue factor.[5] The core area of the plaque is therefore highly thrombogenic.

## STRUCTURE AND DYNAMICS OF THE PLAQUE CAP AND OVERLYING ENDOTHELIUM

The plaque cap has a lattice of collagen within which there are smooth muscle cells. The cap structure is adapted to have high tensile strength, and collagen is constantly replaced and remodeled. In many plaque caps, however, there are empty lacunae, and there is evidence that within the cap smooth muscle cells are undergoing death by apoptosis.[6] Studies of atherosclerosis using scanning electron microscopy[7] show that in normal arteries and over early plaques (fatty streaks) the endothelium is intact without denudation injury to expose connective tissue matrix proteins.
A structurally intact endothelium, however, can be dysfunctional, and there is ample evidence of abnormal expression of adhesion molecules[8] such as vascular cell adhesion molecule (VCAM) in early atheroma and impaired nitric oxide (NO) production in later disease. The rate of proliferation of endothelial cells in human arteries can be studied by using markers of DNA synthesis *(Table I).* Such work shows that over the surface of a plaque replication is markedly increased, but that there is also enhanced endothelial turnover throughout an artery containing plaques. The endothelium over raised plaques also has focal areas of denudation.[7] These areas are ultramicroscopic, involving only small numbers of endothelial cells, but do allow adhesion of a monolayer of platelets to the arterial wall. Microscopic foci of endothelial loss are the rule rather than the exception over advanced plaques. Observational studies in both human and animal models of atherosclerosis link the focal endothelial denudation to the presence in the vicinity of activated macrophages.[7]

| ENDOTHELIAL CELL REPLICATION |
|:---:|
| **Normal artery** |
| No atherosclerosis <1 per 1000 |
| **Atherosclerotic artery** |
| Normal segment 5.7±1.6 per 1000 (n=12) |
| Over plaque 18.9±5.7 per 1000 (n=12) |

*Table I.*

## PLAQUE VARIATION

While it is possible to describe the archetypal type IV and Va plaque, there is a huge structural variation even within one artery. Plaques have an inflammatory phase with a high density of activated macrophages, but this can burn out and leave the lipid core without a rim of foam cells. The size of the lipid core may vary from less than 10% to more than 70% of the overall plaque volume; the fibrous cap may be thick or thin. Plaques that appear yellow on naked eye inspection at autopsy or at angioscopy are those with thin caps and large cores. A small subpopulation of plaques are entirely fibrous without any lipid core (type Vc).[9] While these plaque variations may reflect in part different time points in the evolution of the lesion,[10]

there is some evidence that the risk profile of an individual has an influence.[11] Plaques with a large lipid core are features of Caucasian males with high plasma low-density lipoprotein (LDL) and low high-density lipoprotein (HDL) levels. Some individuals seem to produce more fibrous lesions, and this may be a feature of younger women.

## CELLULAR CHANGES IN THE EVOLUTION OF PLAQUES

The basic premise about the pathogenesis of plaques is that they are local inflammatory lesions in response to the deposition of lipid within the intima. The experimental production of animal lesions that truly resemble the human plaque is achieved only by raising plasma lipids. Such models may be dietary in rabbits, pigs, or primates, or due to natural gene defects in the rabbit (Watanabe model) or transgenic animals. These models give valuable insights into the mechanisms of plaque formation, but rarely produce clinical disease of the type seen in humans. Plasma LDL moves in and out of the arterial intima across the endothelial surface; within the intima, some LDL is converted into a proinflammatory molecule (*Figure 2*).[12] The modification may take place on the endothelial surface or in LDL bound to proteoglycan and involves minor oxidation.



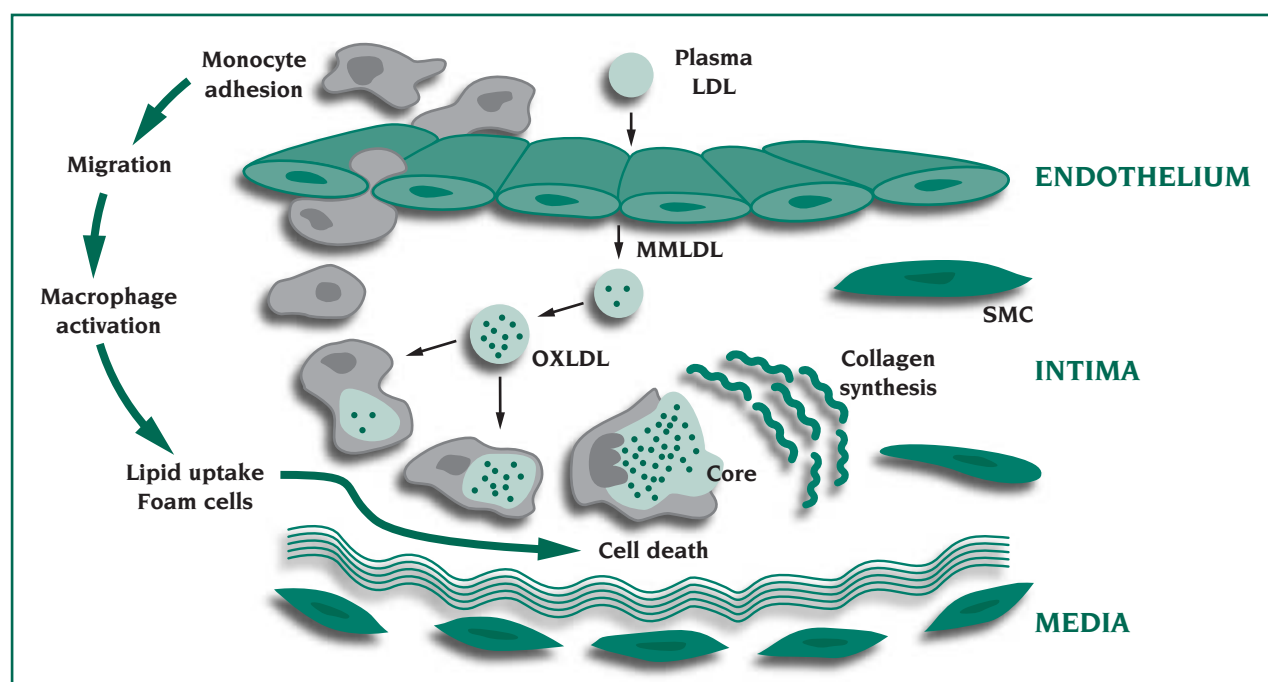*Figure 2. Atherogenesis: plasma low-density lipoprotein (LDL) is modified within the intima to a form that initiates monocyte migration (MMLDL) into the intima. Further oxidation of LDL (OXLDL) levels to a form that is avidly taken up by macrophages to form foam cells. Foam cell death releases lipid to form the lipid core, which then becomes surrounded by collagen synthesized by smooth muscle cells (SMC).*

Modified LDL invokes the expression of adhesion molecules such as VCAM by endothelial cells, leading to monocyte adhesion. Other cytokines and chemotactic proteins such as monocyte chemoattractant protein–1 (MCP-1)[13] and macrophage colony–stimulating factor (MCSF) are also induced,[14] and mediate the migration of monocytes into the intima to establish a population of activated macrophages. Modified LDL within the intima undergoes further oxidation and is now recognized by the scavenger receptor of the macrophage and taken up avidly to form the foam cells of the fatty streak. There is evidence that the fatty streak is a precursor of more advanced plaques, but that by no means implies that all fatty streaks progress. Fatty streaks appear very early in life and can be found in subjects of less than 10 years of age. They occur in the arterial sites, ie, proximal left anterior descending coronary artery, where advanced plaques develop later in life. On the other hand, children in populations where in adults there is minimal advanced plaque formation have abundant fatty streaks. Fatty streaks may be a heterogeneous entity in which some, perhaps those whose foam cells are of macrophage type, progress while others whose foam cells are smooth muscle in type do not progress. Lipid uptake by macrophages is thought to be almost entirely due to scavenger receptors recognizing oxidized LDL. The normal function of these receptors is the elimination of cell debris, for example, alveolar macrophages clear the lung parenchyma. Lipid uptake by smooth muscle cells is due to a low-level expression of the conventional LDL receptor; catabolism of LDL to cholesterol and its esters occurs in the cytoplasm itself. Gene deletions in transgenic models of atherosclerosis have shown how essential growth factors are. If the scavenger receptor is removed,[15] animals are resistant to atherosclerosis, but susceptible to infection. MCP-1 and MCSF knockout also confers resistance to atherosclerosis.

## THE FORMATION OF THE ADVANCED PLAQUE

Lipid core formation is a key step in plaque progression. The core lipid is in large part derived from the cytoplasm of macrophage foam cells that have died *(Figure 2).* Macrophage death is thus a vital step in core formation and occurs by several mechanisms. Apoptosis within macrophages has been shown by a variety of techniques.[16] The macrophages within the core area are highly activated and produce reactive oxygen species, which lead to lipid degradation to produce highly toxic products causing necrosis.

The macrophages can be shown to be synthesizing DNA by the presence of Ki67 and proliferating cell nuclear antigen (PCNA). It is less clear whether this indicates macrophage cell division or cell repair or even preparation for apoptosis.

A proportion of the core lipid is thought to be derived from LDL, which binds to proteoglycans within the intima and then degrades without having passed through the macrophage cytoplasm.
Another component in core formation is the creation of a space with the intima to contain the extracellular lipid. This may be in part passive, with the tissues being pushed apart, but there is also more active destruction of the connective tissue. Reactive oxygen species and cytokines such as tumor necrosis factor α (TNF-α) will induce metalloproteinase production by macrophages, and there are observational human data showing the binding of such enzymes to the connective tissue on the core edge.[17] The fibrous capsule has to develop contiguous with the core, otherwise the plaque would have no solid structure and fall apart. The human coronary intima contains smooth muscle cells that have migrated to this site in fetal life. Once advanced to the lipid core stage, the plaque is a soup of cytokines and growth factors produced by endothelial cells, macrophages, and smooth muscle cells themselves.[18] Many of these factors regulate collagen synthesis in a positive or negative way. Once endothelial denudation has occurred over the plaque, platelets are the source of an additional growth factor, platelet-derived growth factor (PDGF), but this is also produced by the other cells in the plaque. The plethora of growth factors for smooth muscle cells means it is difficult to define which are most important or rate limiting. Blocking PDGF and basic fibroblast growth factor (bFGF) inhibits proliferation after experimental vessel injury, suggesting they have a key role.[19]

## Calcification in plaques

The introduction of computed tomography fast scanning has brought to the clinician's attention the fact that plaques commonly calcify (AHA Type Vb). Two morphological patterns of plaque calcification exist. Within macrophage/lipid-rich areas punctuate areas of calcification develop, which coalesce to form sizeable (2-3 mm) rounded masses. The second form of calcification develops as plates within smooth muscle/collagen-rich areas of the plaque.
Both macrophages and smooth muscle cells undergo a phenotypic change to become akin to osteoblasts

and osteoclasts expressing matrix metalloproteinase-9 (MMP9 = gelatinase B), osteopontin, osteonectin, etc. The presence of calcification in plaques is age-related and in subjects over 80 may be widespread within the coronary intima with relatively minor plaque formation. Medial calcification (Mönckeberg's sclerosis) is unrelated to atherosclerosis and is rare in coronary arteries. The extent of coronary calcification, particularly in subjects under 60 years of age, will be a measure of the number of plaques present and thus of the risk of developing clinically expressed disease.

### Plaque neovascularization

Although the adventitia contains numerous vessels, the normal coronary intima and media are avascular. Once the intima becomes thickened by the formation of plaques, new capillaries begin to extend into the media from the adventitia to enter the base of the plaque.[20] New capillaries also enter the plaque from the main arterial lumen. The stimulus to this vascularization is relative hypoxia in the vessel wall simulating vascular endothelial growth factor production. The new capillaries often become sinusoidal at the base of the lipid core and extravasation of red cells is common, leading to the deposition of some iron pigment. It is uncertain whether neovascularization is good, bad, or neutral with regard to plaque progression. The transmedial vessels express adhesion molecules such as VCAM very strongly and may provide a route for the entry of monocytes into the plaque.

### Plaque inflammation

Every plaque at some stage has an inflammatory phase,[21] based on the reaction of macrophages and endothelial cells to modified LDL. Surgeons who view the atherosclerotic plaque from the epicardial surface in life are impressed by the red angry appearance with a multitude of engorged adventitial blood vessels. Within the plaque itself, activated macrophages have undergone phenotypic changes to become producers of TNF-$\alpha$, metalloproteinases, and interleukins. Class II major histocompatibility complex (MHC) antigen expression is invoked on smooth muscle cells suggesting immune reactions are present.[22] T lymphocytes are present in considerable numbers in plaques intermixed with the macrophages. A range of functions for lymphocytes have been postulated based on observations in human plaques and by use of tissue culture. Macrophages express the CD40 receptor[23] and T lymphocytes its ligand—the result is

macrophage production of tissue factor and proteases. Lymphocytes expressing perforin and granzymes are present, potentially having a cytotoxic role. Within plaques there is an immune reaction to oxidized LDL, initiated by T cells and macrophages, and continued by B lymphocytes and plasma cells in the adventitia to produce autoantibodies. Germinal lymphoid centers may develop in the adventitia and perivascular tissue. Finally, the T lymphocyte produces interferon $\gamma$ and TGF$\beta$ to exert an inhibitory influence on collagen synthesis by smooth muscle cells.

### Fibrinogen and the plaque

Epidemiological studies have shown that elevated plasma fibrinogen levels are a risk factor for the development of ischemic heart disease. One explanation is an enhancement of the thrombotic processes and the increase in plasma viscosity. There may be a more fundamental role in the evolution of the plaque. Fibrinogen moves in and out of the arterial intima; within the plaque, however, tissue factor is present and some thrombin generation occurs, leading to fibrin formation. This process will be a stimulus to smooth muscle proliferation, although active removal of fibrin by the generation of plasmin may exert a natural check on the process. Inspection of the intima of the human atherosclerotic aorta shows a small subpopulation, known as gelatinous plaques. These appear red or brown in color and unlike the fatty streak contain large amounts of fibrinogen. Such gelatinous non–lipid-containing lesions may be the origin of the solid fibrous plaque without a lipid core and an alternative pathway to advanced plaque formation.

## ATHEROSCLEROSIS AND CLINICAL SYMPTOMS

Atherosclerosis produces a number of clinical expressions, including acute ischemic episodes related to thrombosis developing on a plaque, chronic obstruction to blood flow by static plaques that encroach on the lumen, and aneurysm formation in the aorta. While apparently being discrete clinical entities, all of these have closely related basic processes.

### The acute coronary ischemic syndromes

The common theme to the acute coronary syndromes (ACS) of unstable angina, regional infarction, and sudden ischemic death is the development of

**The birth, growth, and consequences of the atherosclerotic plaque -** *Davies*
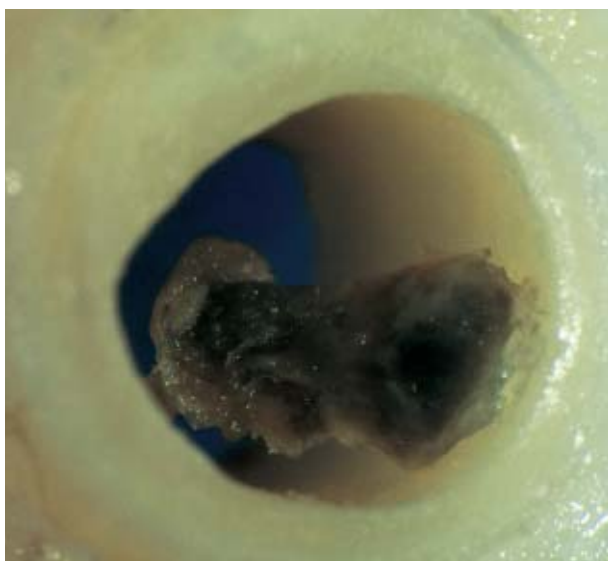


*Figure 3. Human coronary thrombosis due to endothelial erosion. The thrombus is stuck onto the surface of the plaque and is large, but does not occlude the artery.*

thrombosis on a culprit plaque. Thrombosis develops on plaques because of two different processes. One is what has become known as endothelial erosion. As emphasized earlier, focal microscopic loss of endothelial cells over plaques is a regular occurrence. This exposes the underlying connective tissue matrix, allowing some platelet deposition. If larger areas of endothelial cell loss occur, the surface becomes pitted

with large holes into which red cells become entrapped. A coagulation sequence is invoked, which may lead to thrombi a few millimeters across. Such small mural thrombi can, however, enlarge *(Figure 3)* and finally become totally occlusive. This form of thrombosis has been designated as superficial (level 1) injury and is characterized by thrombus adherent to the surface of the plaque. This contrasts with level 2 injury *(Figures 4 and 5)* due to plaque disruption (syn. rupture, fissuring)[24,25] where the cap of a plaque with a lipid core tears, allowing contact of blood in the arterial lumen with the highly thrombogenic interior of the plaque. Thrombus forms inside the plaque, which is distorted and expanded from within. Thrombosis may then extend into the lumen and may ultimately progress to complete occlusion. The magnitude of the cap tear varies: at one extreme it is a fissure or crack, at the other, the whole cap is lost, exposing the core as an ulcer. Plaque disruption is therefore a stimulus for thrombosis, which varies considerably in intensity.

### Repair after plaque thrombosis

Episodes of plaque thrombosis undergo a repair process. This involves natural lysis of thrombus and what is often known as "passification." In this ill-understood process, exposed collagen and the surface of existing thrombi become far less attractive to platelets. One mechanism is that proteoglycans (natural heparinoids) bind to the exposed surface.



*Figure 4. Human nonoccluding coronary thrombosis due to plaque disruption. There is an element of thrombus both within the lipid core itself and that projects into the lumen via a break in the plaque cap (arrow).*



*Figure 5. Occluding coronary thrombosis due to plaque disruption. There is a bilobed mass of thrombus, one part in the plaque another occluding the lumen. The two lobes join through the disrupted cap (arrow).*

Any residual thrombus invokes smooth muscle cell division within the plaque, with the formation of new collagen. The proliferating smooth muscle cells have a characteristic irregular storiform arrangement often known as enhanced or accelerated healing.[26] The end result is that irregularities in the plaque outline are smoothed out, the cap repaired, and the core at least partially obliterated by new collagen over some weeks. The more irregular the outline of the residual stenosis on angiography, the greater is the risk of a further thrombotic episode at the site. Repair of episodes of plaque thrombosis have a very variable outcome. The artery may remain totally occluded, there may be multiple new small vascular channels within the original artery lumen, or there may be a residual single lumen with stenosis ranging from little change from its original state to chronic high-grade stenosis.

## Frequency of plaque thrombotic episodes

Plaque disruption was initially regarded as a major event, which was inevitably associated with acute clinical episodes. This is now recognized to be an oversimplification. Detailed pathology studies in which the whole coronary artery tree has been examined specifically to find morphological evidence of recent thrombotic events show that in subjects with unstable angina/acute myocardial infarction, although there is one major culprit lesion, more minor thrombi are present at other sites. In subjects with coronary atherosclerosis, but who die of an unrelated cause, autopsy shows that up to 10% have had a recent minor plaque disruption. Plaque disruption is therefore now seen as an integral part of atherosclerosis progression and not solely as a cause of acute clinical events.

## Unstable angina

The essence of unstable angina is pain and transient ECG changes at rest. However, the term covers a very wide spectrum of severity and risk. Crescendo angina (type IIIB) in the last 48 hours is the form most associated with a significant risk of sudden death or myocardial infarction and therefore has been most studied.

Angiographic studies compared the lesions regarded as responsible for stable and unstable angina.[27] The former were associated with smooth stenoses (type I), while the latter were associated with eccentric ragged stenoses often with overhanging edges (type II). A seminal pathology study showed type II angiographic lesions were disrupted atherosclerotic plaques.

The torn cap and thrombus project into the arterial lumen, but antegrade blood flow is still present. The frequency of demonstrable intraluminal thrombi on angiography in unstable angina was between 20% and 50%, but this figure rose to around 70% once angioscopy with its greater sensitivity came into clinical use.[28] Atherectomy has also made contributions[29]; samples taken from the culprit lesions of unstable angina compared with the material from plaques causing stable angina had a higher frequency of thrombus and macrophages. However, the techniques of angioscopy and atherectomy showed that the differences between plaques causing unstable and stable angina are of degree rather than absolute. Thrombus is not found in all lesions causing unstable angina and, conversely, is present in up to 10% of lesions responsible for stable angina. The absence of thrombus in a proportion of unstable cases is in part due to the random selection of fragments that are taken at atherectomy and in part time-related. The natural history of the lesions responsible for unstable angina is to progress to thrombotic occlusion or to resolve and heal. Atherectomy samples that are taken in this resolving period will show accelerated smooth muscle proliferation rather than thrombotic material.[26,29]

Thrombotic material in plaques related to stable angina reflects the importance of subclinical episodes of thrombus in the growth of plaques and the progression of stenosis. The typical lesion of unstable angina *(Figure 4),* with its mass of thrombus projecting into the lumen, causes transient ischemic episodes by three possible mechanisms. The first is that of intermittent local arterial spasm. Many of the plaques causing unstable angina are eccentric, ie, there is an arc of normal vessel wall still present that is able to contract and reduce the lumen size. Second is the potential for intraluminal thrombi to wax and wane in size over quite short periods of time and cause intermittent occlusion.[30] The third mechanism is of distal embolization of platelets.[30,31] The surface of any thrombus that protrudes into the arterial lumen is covered by a layer of activated platelets with high levels of expression of the IIb/IIIa receptor. Aggregates of these platelets are washed off the surface of the thrombus and impact in small intramyocardial arteries. Such emboli are associated with microscopic foci of myocyte necrosis. The increasing use of troponin T estimations as a marker of myocyte necrosis in vivo in unstable angina has confirmed that elevated levels occur in a significant proportion of cases and act as a prognostic marker.

What is perhaps surprising is that the lesions of unstable angina persist for some days. This implies that the exposed thrombus is in a balanced state in which the tendency for thrombotic progression is balanced by factors promoting healing.

Factors favoring progression to occlusion include large plaque events with massive exposure of the core, low blood flow, and high viscosity, while, conversely, high blood flow, small plaque events, and high lytic potential will favor resolution.

## Acute myocardial infarction

The great majority of regional areas of myocardial necrosis (infarction) are due to coronary atherosclerosis complicated by thrombosis.[32] Transmural regional infarct is due to thrombotic occlusions that develop over a relatively short time period, often in an artery in which the myocardium is not protected by the prior development of collateral flow. The thrombi develop as a result of plaque disruption in around three quarters of cases, the remainder being due to plaque erosion. The magnitude of the underlying plaque event varies widely. At one extreme there is a very small plaque tear—the word fissure is often used for these events. The degree of thrombotic response is disproportionately large in the lumen and there is no significant intraplaque thrombus. This is the end of the spectrum every clinician would like to treat—lysis removes the intraluminal thrombus and the artery can be reopened, often to leave no residual stenosis. In the mid-range of the spectrum are cases with a bilobed mass of thrombus which is part within and expanding the plaque and part occluding the lumen *(Figure 5)*. At the extreme end of the spectrum are plaques that have lost their whole cap, and the lumen is occluded by a mass of thrombus, cholesterol, and fragments of the plaque cap. Such plaque disasters would be anticipated to be best reopened by primary angioplasty.

The thrombotic response to plaque disruption occurs in stages *(Figure 6)*, although these may be passed through rapidly.[25] The initial stage is within the plaque itself as blood comes into contact with the lipid core. Much of this intraplaque thrombus is rich in platelets, although red cells can also be prominent. A second stage consists of more densely packed fibrin-rich thrombus in and around the rupture site itself. Finally, the occluding thrombus has a loose network of fibrin with intermeshed red cells, but has a minor platelet component. It is understandable that fibrinolytic therapy will be effective against this third
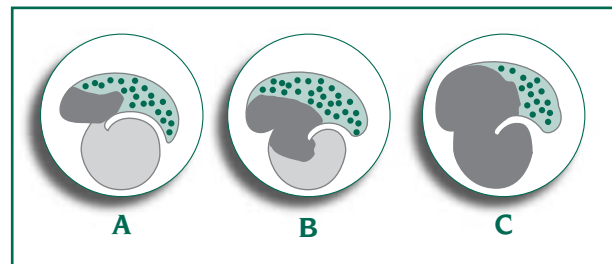


**Figure 6.** *Stages in the evolution of thrombus (black) following plaque disruption through predominantly intraplaque thrombus (A) to mural thrombus (B) projecting into the lumen, but not causing a total cessation of flow to occlusive thrombus (C). Green = lipid core.*

and occluding stage thrombus, but this will expose the more resistant thrombus in the plaque itself. The angiographic studies of DeWood[33] and Stadius[34] clearly showed the dynamic nature of the thrombi causing acute regional infarction. The closer in time to the onset of regional infarction the angiogram was carried out the higher was the frequency of a totally occluded artery. However, spontaneous reopening was not a rare event. Further light on the dynamics was shed by Fulton and Sumner.[35] Radiolabeled fibrinogen was given to patients with acute infarction as soon as possible after the onset of pain. In those who came to autopsy, the distribution of the radiolabel in the thrombus was carefully analyzed. The head of the thrombus was not labeled, indicating it predated the onset of infarction. The tail of the thrombus where it had extended distally was, however, labeled, indicating thrombus continued to propagate downstream after infarction had occurred. This phenomenon explains the quixotic view held by some pathologists in the 1980s that thrombosis was entirely secondary to infarction.

Nontransmural regional infarction also has a close relationship to coronary thrombosis, but has a different timescale than transmural infarction. Transmural infarction is characterized by the onset of necrosis that has occurred over a relatively short time span, certainly less than 12 hours. In contrast, nontransmural infarction is often the result of coalescence of multiple subendocardial foci of infarction that differ in age by as much as 7 to 10 days. It often follows a period of unstable angina and is probably due to multiple small foci of myocardial necrosis occurring because of microemboli or intermittent complete occlusion. Another element in the production of nontransmural rather than transmural infarction is the existence of some prior collateral flow to the region.

### Ischemic sudden death

Sudden death within a short period of the onset of symptoms or without any warning symptoms has a heterogeneous pathology. A high proportion of cases have a recent coronary thrombotic event based on disruption or erosion. Of these, a proportion have a complete thrombotic occlusion and would have been candidates for developing an acute regional infarct had they survived. The majority of thrombi responsible for sudden ischemic death are, however, not occlusive, and the arterial lesions and their postmortem angiograms are identical to those seen in living subjects with crescendo unstable angina. About half of those with nonocclusive thrombi have intramyocardial platelet embolization.[31] The proportion of sudden ischemic deaths with a recent thrombotic event ranges from 50% to 75% in different pathology series.

## THE MECHANISM OF PLAQUE DISRUPTION AND EROSION

Endothelial erosion is in most cases a reflection of the proximity of activated macrophages.[36] In addition, T lymphocytes and smooth muscle cells expressing class II antigens in the area provide evidence for a local immune inflammatory damage. The endothelial loss may be due to apoptosis or to the cells being cut free from their adhesion to the subendothelial matrix by proteolytic enzymes produced by macrophages. Plaque disruption has a more complex pathophysiology. Pathology studies[37,38] have shown the characteristics of plaques that have undergone recent disruption:

- A large lipid core occupying more than 50% of total plaque volume;

- A high concentration of macrophages in the cap and in the area around the lipid core;

- High levels of the expression of tissue factor and metalloproteinases by macrophages;

- Low density of smooth muscle cell in the cap;

- Thin plaque caps with disorganized arrangement of collagen.

While these characteristics, implying as they do a plaque with active inflammatory changes, are found in disrupted (unstable) plaques, they are also found to some degree in many other intact plaques in the same artery. Plaques with a concordance of all the features listed above, but in which there is no current disruption, can be regarded as *vulnerable,* that is, at a high risk of a future event. The rupture of a plaque cap is essentially mechanical. The tearing depends on the interplay between the force exerted on the plaque cap and the innate mechanical strength of the cap. Both sides of this equation have been widely studied.

The microanatomy of the plaque has a profound influence on the distribution of circumferential wall stress in systole.[39,40] The lipid core being easily compressed cannot carry a load. The load is redistributed and falls very heavily on the plaque cap. Stresses up to eight times normal become concentrated on focal points in the cap. The usual point of maximum stress is at the junction of the cap and the normal vessel wall, a site at which cap tears are common. Use of finite analysis computer predictions suggests that a thin cap and eccentric plaques that do not cause stenosis will enhance the tendency of high loads to be exerted on the cap. The other side of the equation is the innate mechanical strength of the plaque cap. In vitro testing of plaque caps by stretching to break point shows that a loss of the normal arrangement and reduced concentration of collagen lead to weakness.[41] Cap material is not uniform in its strength with focal weak areas more likely to initiate tearing than other areas. The presence of macrophages in the cap tissue is also associated with weakness.

Plaque cap tears therefore represent both increased load on the tissue and a reduction in cap tensile strength. There is probably an element of chance in that, if the point of maximum stress happens to fall on a focal weak point in the cap, a tear is initiated, which may then propagate. The work of Libby[42] has highlighted the concept *(Figure 7, next page)* that the plaque cap is a dynamic structure in which smooth muscle cells synthesize the connective tissue matrix including collagen. Collagen synthesis is diminished by interferon gamma produced by lymphocytes and is one mechanism by which the cap can be weakened by an inflammatory process.

Smooth muscle cells are also vulnerable to spontaneous death by apoptosis, a process accelerated by the proximity of macrophages.[43] Increased degradation of collagen also occurs in the cap. Metalloproteinases are a family of enzymes of which at least 14 are now recognized, each having a different molecular weight and affinity for degrading different components of the connective tissue matrix. They are all secreted by the cell into the tissues as an inactive form, with activation occurring due to the action of plasmin, mast cells, or other metalloproteinases. Observational studies of human plaques[17] show that many of these proteases can be demonstrated by immunohistochemistry.
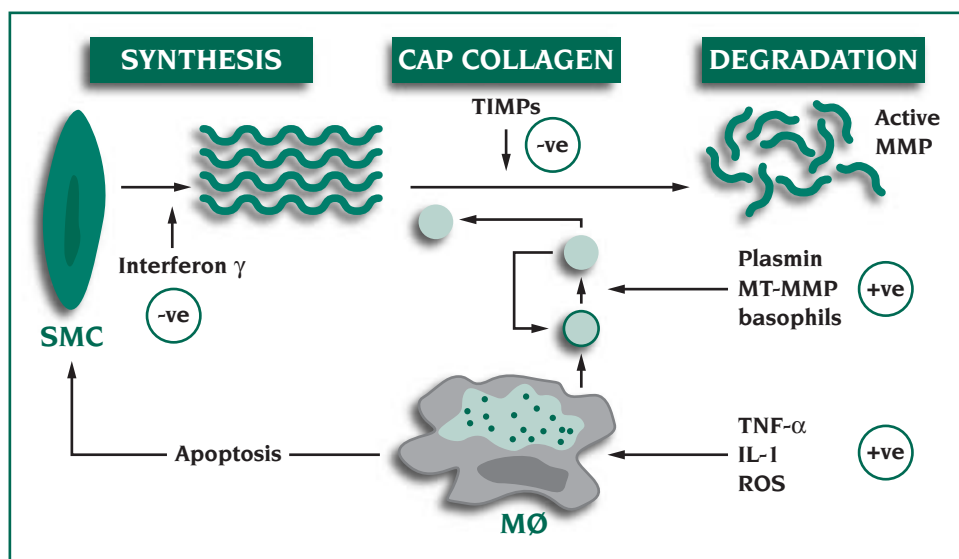
*Figure 7. Plaque cap dynamics. Collagen is synthesized by smooth muscle cells, but degraded by metalloproteinases. Macrophages release metalloproteinases in an inactive form, which is activated by plasmin, other matrix metalloproteinases, or mast cells to begin degradation of mature collagen to low-molecular-weight products.*

*Abbreviations: IL-1, interleukin-1; MØ, monocyte/macrophage; MT-MMP, membrane-bound matrix metalloproteinases; ROS, reactive oxygen species; SMC, smooth muscle cell; TIMPs, tissue inhibitors of metalloproteinases; TNF-α, tumor necrosis factor α; +ve, positive; -ve, negative.*

Metalloproteinases are produced by both smooth muscle cells and macrophages. Smooth muscle cell production of gelatinase A and membrane-bound metalloproteinase is probably concerned with cell migration. Macrophage production of MMP9 (gelatinase B), MMP3 (stromelysin), and MMP1 (collagenase) is concerned with cap matrix destruction. Immunohistochemistry on human plaque tissue is limited by the fact that antibodies usually recognize both the active and inactive forms of the metalloproteinase and that tissue inhibitors of metalloproteinases exist (TIMPs). The development of a technique in which the cap tissue was laid on a sheet of gelatin in vitro and dissolution identified confirmed, however, that active matrix breakdown was occurring in plaques due to an excess of the active protease.[44] The maximal sites of enzyme activity coincide with areas of high stress and with macrophage accumulations. Once again, therefore, this suggests cap destruction is a reflection of inflammatory activity. In vitro studies of macrophages show that a range of substances including TNF-α, interleukin-1 (IL-1), reactive oxygen species (ROS), and oxidized lipid will upregulate the expression of metalloproteinases by macrophages.

### THE VULNERABLE PLAQUE CONCEPT

A variable proportion of all the plaques in an individual will have large cores, thin caps, and a high inflammatory component. It is these plaques that are *vulnerable*, ie, at risk of causing a new acute ischemic event in the future. Therefore, the risk of a new event depends not so much on the total number of plaques

as on the number of vulnerable plaques. Some subjects have few lipid-rich plaques with large cores while others have most of their plaques with large cores. The reasons for this variation are unclear but risk factors probably play a role.[1-3,11] There is one important clinical consequence of the vulnerable plaque concept. The properties conferring vulnerability on a plaque bear no relation to the degree of coronary artery stenosis.[45] A high proportion of vulnerable plaques are angiographically invisible, with the corollary that the angiogram does not, and cannot, give any information on the risk or site of future coronary thrombotic events.

### Inflammatory and infective enhancers of the risk of acute events

There has been intense interest in raised levels of systemic inflammatory markers, both in the acute phase of unstable angina and in subjects with currently stable ischemic heart disease. What is impressive is the similarity of the findings with a heterogeneous group of inflammatory markers including fibrinogen, C-reactive protein (CRP), neopterin, the erythrocyte sedimentation rate (ESR), and plasma albumin.

Elevated systemic markers in subjects with chronic ischemic heart disease do indicate future risk. In the Physicians Health studies,[46] both CRP and ICAM levels in the highest quartile of the population increase the risk of a future myocardial infarction by a factor approaching three. The level of the CRP, however, is not very high even in the upper quartile group, and the estimations are good predictors on a

population rather than an individual basis.
The reasons for these minor elevations in inflammatory markers in chronic disease are contentious. The likely answer is that they in some way indicate activity of the atherosclerotic process overall. The majority of subjects with atherosclerosis have a far greater plaque volume in the aorta to release inflammatory markers than in the coronary arteries. Up to a third of individuals with coronary disease have thrombus in the abdominal aorta, which will inevitably cause platelet activation. On a population basis, extensive aortic atherosclerosis will be a surrogate for extensive coronary disease.

Subjects with unstable angina are regarded as having a single culprit plaque; this is an oversimplification, and there are often other plaques with small thrombi present. It is possible that a systemic enhancement of inflammatory activity is a trigger for increasing plaque activity. There is epidemiological evidence for an undue preponderance of viral infections before acute ischemic episodes. A systemic inflammatory reaction may nonspecifically elevate cytokine expression within plaques,[47] an example may be the increased risk of ischemic heart disease in *Helicobacter* infection.

Chlamydial infection is another putative enhancer of plaque inflammatory activity and therefore of the risk of acute ischemic events. Up to 40% of individuals with atherosclerotic plaques have chlamydial colonization of their plaques.[48] The organism is present in large numbers and capable of division. The cell most frequently infected is the macrophage, both in its foam cell form deep within the plaque and in monocytoid cells close to the plaque surface. Some workers also report *Chlamydia* within smooth muscle and endothelial cells. The chlamydial organisms are thought to enter the plaque by what Libby has referred to as the "Trojan Horse" mechanism.[49]
Monocytes pick up the organism in the upper respiratory tract and enter the circulation where they then adhere to the endothelium over plaques and migrate inward. This implies that either the infected monocytes are selectively recruited by plaques or that the infected cells are sequestered at many sites, but only in the plaque is there a milieu in which the organism proliferates. The question is whether chlamydial infection of plaques is of any consequence or whether the organism is a harmless tenant. Chlamydial serology has provided some evidence[50] that infection increases the risk of acute ischemic events, and trials to eradicate the infection by antibiotics are under way in the hope of reducing acute

events. There are mechanisms by which *Chlamydia* within plaques would upregulate inflammation and therefore increase the risk of events. *Chlamydia* both produce their own heat shock proteins (HSP) and induce the formation of HSP 60 by macrophages, which in turn increases IL-1 and TNF-$\alpha$ production within the plaque.

## THE GENERATION OF CHRONIC HIGH-GRADE CORONARY STENOSIS

Before coronary angiography became widely available, coronary stenosis was regarded as the result of linear plaque growth. However, serial angiography in subjects with chronic coronary disease shows that progression is stepwise, not linear with time.[51,52] New high-grade stenotic lesions appear de novo between angiograms, and while high-grade stenotic lesions do progress, many minor lesions remain static for long periods. A further paradox needing explanation was that pathologists found plaques in arteries that had been angiographically normal in life.

### Arterial remodeling and compensatory dilatation

The paradox that angiographically normal arteries contained plaques was resolved by the seminal work of Glagov et al.[53] They showed that as atherosclerosis developed the artery increased its external dimensions to accommodate the plaque while conserving the lumen dimensions. This process of remodeling of the artery wall has become known as compensatory dilatation and involves a diffuse remodeling of the whole artery with a rearrangement of the medial smooth muscle cells to create a larger total cross-sectional area. The media is therefore now seen as a dynamic, not an inert, structure.

The advent of intravascular ultrasound confirmed the process of arterial remodeling in atherosclerotic arteries.[54] Remodeling is plaque-specific rather than a property that one patient has to a greater degree than another. Within one coronary artery, one plaque may be associated with remodeling to a degree that the lumen size is unaltered, while within a few centimeters another plaque has not invoked remodeling and angiographic stenosis is present. The terms adequate and inadequate compensatory dilatation have come into use to reflect whether the plaque has or has not invoked sufficient remodeling to prevent stenosis.

Intravascular ultrasound studies have suggested that negative remodeling occurs, ie, that the external dimensions of the artery have decreased at a plaque site. Pathology studies find this to be rare outside postangioplasty stenosis, where adventitial fibrosis externally constricts the artery. One of the difficulties of intravascular ultrasound is the reference sites used to compare the lumen and vessel cross-sectional area at the plaque site. Pathology studies show that some dilatation of the morphologically normal arterial segments proximal and distal to sites of stenosis occurs—such dilatation will lead to the spurious interpretation that the stenotic area has a reduced vessel cross-sectional area.

The evolution of high-grade stenosis now has to be seen as a balance between plaque growth and the ability of the vessel to increase its cross-sectional area. The great majority of segments of high-grade coronary stenosis have a failure of remodeling rather than excessive plaque growth. This being so, there must be a reason for the failure, but for the moment this is unclear. Pathology studies of stenotic segments in coronary arteries provide some explanations. The use of dyes such as Sirius red, which binds to collagen and can be viewed under polarized light allows the underlying morphology of plaques to be more clearly appreciated. In effect an "archeological dig" can be carried out to allow the identification of episodes of healed plaque disruption. The breaks in the original cap can be identified by the deposition of finely arranged collagen with local increase in the smooth muscle cell densities. Such studies suggest that with segments of high-grade stenosis over 50% by diameter on angiography, 68% have had an episode of healed disruption. Such data would explain the sudden appearance of stenotic segments between annual angiograms and highlight that subclinical acute thrombotic events are an important mechanism in chronic disease progression. Sudden stimulation of smooth muscle proliferation within a plaque by thrombus would cause a rapid increase in plaque growth, the speed of which may be too rapid for adequate compensatory dilatation to occur.

## AORTIC AND CAROTID ATHEROSCLEROSIS

Chronic plaque ulceration and aneurysm formation occur in large artery atherosclerosis. The plaques of the aorta and its major branches may be up to 2 or 3 cm in length, but have exactly the same characteristics as coronary plaques with lipid cores and caps. When these plaques rupture, the whole cap is lost and the cholesterol core is washed out to leave an ulcer crater. The crater fills with thrombus,[55] which remains mural due to the high-flow rates in such large caliber arteries. The thrombus will ultimately be removed and the crater become reendothelialized, but this is a drawn out process and distal emboli of cholesterol crystals and platelet aggregates can occur over long periods. The phenomenon is best recognized in the carotid arteries as a cause of transient cerebral ischemic attacks, but is also important in the steady reduction by embolic occlusion of the number of small arteries in the lower limbs.

The abdominal aorta below the renal arteries is maximally involved in most subjects with atherosclerosis. It is at this site that aneurysms develop when plaque formation becomes confluent. While these aneurysms are clearly related to atherosclerosis, they are familial outside the conventional risk factors for atherosclerosis—this implies there are additional genes involved. The current view is that the extensive plaque formation is associated with movement of activated macrophages into the media and also with an intense periadventitial inflammatory response to oxidized lipids. Destruction of the elastin and collagen of the media and adventitia by metalloproteinases released in the inflammatory process occurs.[56] The separate familial tendency is thought to involve polymorphisms in genes that influence metalloproteinase activity.

## THE TREATMENT OF ATHEROSCLEROSIS

The epidemiological relation between elevated plasma LDL levels and the risk of developing ischemic heart disease led to attempts to influence the progression of the disease by lowering plasma lipids. Once the statins were produced this attempt became a practical proposition. Angiographic studies setting out to demonstrate regression, ie, a reduction in stenosis, proved a disappointment. The degree of regression obtained, while statistically present, was of a degree too small to be clinically significant. What was impressive, however, was that the rate of appearance of new angiographic lesions was reduced. Angiographic studies have been overtaken by acute event trials. Studies of lipid lowering in both a primary and secondary context and across a wide range of lipid levels show it is possible to significantly reduce the risk of future events. The question arises

over the mechanism. The basis of acute ischemic events is thrombosis initiated by erosion or disruption. The logic is therefore that lipid lowering must reduce the frequency of erosion or disruption by improving plaque stability (lowering vulnerability). The mechanism has been suggested by the use of animal models of atherosclerosis induced by lipid-rich diets and in the Watanabe rabbit. A remarkably consistent message has emerged in both primates and rodents on whether lipid is lowered by a statin or by altering the diet.[57]

The changes within the plaques are:

• A reduction in the number of macrophages;

• A reduction in the products of macrophage activation such as MCP-1, metalloproteinases, and tissue factor;

• An increase in smooth muscle cell density;

• An increase in collagen;

• Some reduction in plaque lipid content;

• A modest reduction in plaque size.

These are just the changes that would be anticipated to produce a solid, less inflammatory, more stable plaque. Confirmation that similar changes occur in humans may come from ongoing intravascular ultrasound studies aiming to show reduction in lipid core sizes in patients whose lipids are lowered. The clear data on the effect of lipid lowering on plaque morphology do not preclude additional systemic effects of lipid lowering both by statins and diet. There is clinical evidence of a rapid improvement in endothelial function—this would be in accord with the concept that a reduction in macrophage inflammatory activity may be important in endothelial damage. Women and diabetics have more endothelial erosion as a cause of acute events, but the CARE Study[58] shows they gain as much benefit from lipid lowering as males. In vitro studies suggest that some statins have potentially beneficial additional properties such as antiplatelet and antioxidant activity, but there is little solid evidence these are important in vivo.

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### THREE KEY QUESTIONS

There have been immense advances in the understanding of atherosclerosis. The most important has been the realization that lipid lowering can alter the natural history of the disease. Yet the fact remains that the treatment arms of lipid-lowering trials continue to show very significant numbers of acute ischemic events. Why have we not reduced the risk more? A 30% reduction in risk is good, but 100% would be better. This leads Stuart Cobbe to ask: **"Why does lipid lowering only reduce CHD events by half?"** We understand that patients vary in their risk of acute events, but how can we detect those at the highest risk in order to target more aggressive therapy? Will inflammatory markers help and do they indicate nonlipid-derived factors are also driving atherosclerosis? With this in mind, Robert Califf asks: **"How can we identify patients at high risk of CHD events?"** Finally, unstable angina is still a common disease manifestation. How can those at the highest risk be identified and managed to prevent sudden death and/or infarction? This concern is echoed by Andrew Tonkin and Derek Chew, who pose the question: **"How can we stratify patients with unstable angia and determine optimal therapy?"**

Opinions expressed are those of the author and not the institution.

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# Atheroma

*Expert Answers to Three Key Questions*

## ⓵

## Why does lipid lowering only reduce coronary heart disease events by half?

*S.M. Cobbe*

## ⓶

## How can we identify patients at high risk of coronary heart disease events?

*R.M. Califf*

## ⓷

## How can we stratify patients with unstable angina and determine optimal therapy?

*A. Tonkin, D. Chew*

# Why does lipid lowering only reduce coronary heart disease events by half?

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*Epidemiological data suggest that a prolonged difference of 0.6 mmol·L⁻¹ (approximately 10%) in total cholesterol results in an average reduction in relative risk of coronary heart disease (CHD) of 27% in cohort studies and 38% in international studies. Five large, randomized controlled trials of lipid reduction by the HMG-CoA– reductase inhibitors (statins) have shown unequivocal clinical benefit in primary and secondary prevention. The crude risk reduction per 0.6 mmol·L⁻¹ cholesterol in these trials was 12% to 21% (average 15%), approximately 50% of the epidemiologically predicted effect. If allowance is made for the impact of regression dilution bias, for possible overestimation of the association between cholesterol levels and CHD risk in epidemiological studies, for poor or noncompliance, and for the limited duration of the trials, it is likely that the true benefit of long-term statin therapy will be close to that predicted from cohort studies.*

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The causal association between increased total and low-density lipoprotein (LDL) cholesterol levels and coronary heart disease (CHD) has long been recognized. Since 1994, five large, randomized controlled trials of the effect of lipid reduction by the β-hydroxy-β-methylglutaryl– coenzyme A (HMG-CoA)–reductase inhibitors lovastatin, pravastatin, and simvastatin have been published,[1-5] and have put an end to any doubts as to the clinical benefit of lipid reduction. The data provided by these trials offer opportunities to study whether predictions derived from epidemiological studies on the relationship between the magnitude of total or LDL cholesterol reduction and that of CHD events are realized in practice.

## EPIDEMIOLOGICAL DATA

The relationship between plasma cholesterol and CHD events has been studied in a number of ways. International studies relate the incidence of CHD events to the mean cholesterol levels in the different populations under study. Cohort studies involve the measurement of cholesterol and other risk factors at a baseline examination, following which the incidence of coronary events during a follow-up period is determined. The study population is subdivided by ranking individual measurements of baseline cholesterol, and grouping them, usually into quintiles. The associa- tion between the mean cholesterol values in each quintile and their CHD risk is calculated.

The major epidemiological studies were reviewed and summarized by Law et al,[6] who demonstrated a log-linear relationship between cholesterol and CHD risk. In cohort studies, they calculated that a prolonged difference of 0.6 mmol·L⁻¹ (approximately 10%) in total cholesterol was associated with an average reduction of 27% in CHD relative risk in men.

Mean cholesterol levels in interna- tional studies have varied from 3.8 mmol·L⁻¹ (rural China) to 7.0 mmol·L⁻¹ (Finland),[7] and account for 80% of the tenfold difference in CHD risk between countries. The esti- mated change in risk resultant upon a 0.6 mmol·L⁻¹ difference in choles- terol from 3 international studies covering 17 countries (for men aged 55 to 64 years) was 38% (95% confidence interval [CI], 33% - 42%). In longitudinal surveys, cholesterol reductions of the order of 0.6 mmol·L⁻¹ have been reported over about 5 to 10 years in some communities. This reduction has been associated with a decrease in CHD mortality of around one third.

## REDUCTION IN CHOLESTEROL AND CHD RISK IN CLINICAL TRIALS

Clinical trials of cholesterol reduc- tion have consistently reported

values for baseline and on-treatment total and LDL cholesterol, as well as the incidence of major coronary events, usually defined as nonfatal myocardial infarction plus either fatal myocardial infarction or CHD death. The data in *Table I* illustrate the mean baseline lipid levels in the two recent primary prevention trials, the West Of Scotland COronary Prevention Study (WOSCOPS)[2] and the Airforce/Texas Coronary Atherosclerosis Prevention Study (AF/TexCAPS).[5] Data are also presented for the three secondary prevention trials, the Scandinavian Simvastatin Survival Study (4S),[1] the Cholesterol and Recurrent Events Trial (CARE),[3] and the Long-term Intervention with Pravastatin in Ischaemic Disease study (LIPID).[4] The entry criteria for total cholesterol ranged from 4.65 to 7.8 mmol·L⁻¹ in the primary prevention trials, and from 4.0 to 8.0 mmol·L⁻¹ in the secondary prevention trials, values that cover the majority of cholesterol levels seen in Western societies.

The 5-year risk of CHD death or nonfatal myocardial infarction ranged widely in the placebo groups, from 2.9% in AF/TexCAPS to 28% in 4S. However, despite the near tenfold difference in baseline risk, the relative risk reduction achieved by statin therapy was consistent, ranging from 24% to 40%.

The mean reduction in total cholesterol in the five statin trials ranged from 1.02 to 1.75 mmol·L⁻¹, and in LDL cholesterol from 0.97-1.75 mmol·L⁻¹ *(Table I). Table II* illustrates that the crude reduction in relative risk per 0.6 mmol·L⁻¹ reduction in total cholesterol was 12% to 21% (average 15%), while that for LDL cholesterol ranged from 12% to 22% (average 16%).

Subgroup analyses of the relationship between the extent of cholesterol and risk reduction have been published for the Lipid Research Clinics Coronary Primary Prevention Trial (LRC-CPPT),[8] the Helsinki Heart Study (HHS),[9] as well as for 4S,[10] WOSCOPS,[11] and CARE.[12] These analyses have either used lipid values as a continuous variable in multivariate analysis, or have subdivided the active treatment group

into quintiles according to magnitude of cholesterol reduction, or achieved cholesterol levels. These are essentially data-derived analyses, and should be interpreted with caution.[13] The risk reduction per 10% lowering in LDL cholesterol was estimated as 19% (95% CI, 8%-30%) in the LRC-CPPT,[8] and 23% (95% CI, 1%-44%) in the HHS.[9] Both of these studies had small numbers of end points, hence the wide confidence intervals. The data from 4S indicated a risk reduction of 19% (95% CI, 10%-28%) for total cholesterol and 17% (95% CI, 10%-24%) for LDL cholesterol.[10] The association between cholesterol reduction and risk was curvilinear, but the authors did not attempt to analyze it using a log-linear model. Subgroup analyses from CARE and WOSCOPS did not suggest a linear relationship between lipid lowering and risk reduction. In CARE,[12] lower LDL cholesterol was associated with lower risk down to approximately 3 mmol·L⁻¹, below which no further risk reduction was seen. The subgroup analysis of the pravastatin-treated subjects in WOSCOPS[11] was based on quintiles

| Trial | TC baseline (mmol·L⁻¹) | LDL baseline (mmol·L⁻¹) | ΔTC (mmol·L⁻¹) | ΔLDL (mmol·L⁻¹) | Fatal/nonfatal MI active (%) | Fatal/nonfatal MI placebo (%) | RR (%) |
|---|---|---|---|---|---|---|---|
| **AF/TexCAPS** | 5.71 | 3.89 | 1.15 | 1.08 | 1.7 | 2.9 | 40 |
| **WOSCOPS** | 7.07 | 4.99 | 1.41 | 1.30 | 5.5 | 7.9 | 31 |
| **4S** | 6.75 | 4.87 | 1.75 | 1.75 | 19 | 28 | 34 |
| **CARE** | 5.43 | 3.61 | 1.09 | 0.99 | 10.2 | 13.2 | 24 |
| **LIPID** | 5.67 | 3.90 | 1.02 | 0.97 | 12.3 | 15.9 | 24 |

| | | | |
|---|---|---|---|
| **AF/TexCAPS** | Airforce/Texas Coronary Atherosclerosis Prevention Study | **LDL baseline** | Mean baseline LDL cholesterol |
| **CARE** | Cholesterol and Recurrent Events Trial | **LIPID** | Long-term Intervention with Pravastatin in Ischaemic Disease study |
| **CHD** | Coronary heart disease | **MI** | Myocardial infarction |
| **Fatal/nonfatal MI** | Incidence of coronary heart disease death (fatal myocardial infarction) plus nonfatal myocardial infarction during an average 5-year follow-up | **4S** | Scandinavian Simvastatin Survival Study |
| | | **ΔTC** | Reduction in total cholesterol on active therapy |
| | | **TC baseline** | Mean baseline total cholesterol |
| **LDL** | Low-density lipoprotein cholesterol | **RR** | Risk reduction |
| **ΔLDL** | Reduction in LDL cholesterol on active therapy | **WOSCOPS** | West Of Scotland COronary Prevention Study |

**Table I.** *Changes in total and LDL cholesterol and CHD event rates in statin trials.*

| Trial | RR (%) | ΔTC (mmol·L⁻¹) | RR/Δ0.6TC (%) | ΔLDL (mmol·L⁻¹) | RR/Δ0.6LDL (%) |
|---|---|---|---|---|---|
| **AF/TexCAPS** | 40 | 1.15 | 21 | 1.08 | 22 |
| **WOSCOPS** | 31 | 1.41 | 13 | 1.30 | 14 |
| **4S** | 34 | 1.75 | 12 | 1.75 | 12 |
| **CARE** | 24 | 1.09 | 13 | 0.99 | 15 |
| **LIPID** | 24 | 1.02 | 14 | 0.97 | 15 |

| | | | |
|---|---|---|---|
| **RR** | Risk reduction | **RR/Δ0.6LDL** | Risk reduction per 0.6 mmol·L⁻¹ reduction in LDL cholesterol |
| **RR/Δ0.6TC** | Risk reduction per 0.6 mmol·L⁻¹ reduction in total cholesterol | **Trial acronyms as in Table I** | |

**Table II.** *Relationship between total cholesterol/low-density lipoprotein (LDL) choslesterol reduction and risk reduction in statin trials.*

of LDL cholesterol reduction. There was no mean reduction in LDL cholesterol or in risk in the first quintile. Quintiles 2-5 showed mean LDL cholesterol reductions of 12%, 24%, 31%, and 39%, respectively. The relative risks in comparison to placebo were 1.09, 0.72, 0.53, 0.69, and 0.51 in quintiles 1-5, respectively. There was no significant difference in risk between quintiles 2-5, suggesting either a threshold effect in lipid reduction, or a curvilinear or log-linear relationship between risk and cholesterol reduction.

In summary, the risk reduction per 0.6 mmol·L⁻¹ (approximately 10%)

in cholesterol in the cholesterol-lowering trials represents approximately 50% of the epidemiologically predicted effect.[6] A number of factors, singly and in combination, may account for this apparent discrepancy, and are discussed in the following sections.

## REGRESSION DILUTION BIAS

A single measurement of plasma cholesterol does not afford a particularly accurate index of an individual's cholesterol-related CHD risk.[6,14] Total and LDL cholesterol measurements are subject to random analytical error,

and there are seasonal and long-term variations in lipid levels. Epidemiological studies attempt to measure the relationship between the "usual" cholesterol level and CHD risk. The ideal way of estimating the "usual" cholesterol is to use the mean of multiple measurements taken over a prolonged period. If this is not practicable, an alternative approach is to make repeat measurements in a random sample of the original cohort, and to use the difference between the initial and repeat measurement to correct the mean values for the subgroups of the cohort. This process allows for the phenomenon of regression to the mean, by which values that were initially higher than the "usual" cholesterol for an individual as a result of random variation will be lower on repeat testing, while values that were initially lower will be higher on repeat testing. The impact of such a correction is illustrated in *Table III,* based on data from the British United Provident Association (BUPA) cohort study by Law et al.[15] A group of 21515 men was screened for cardiovascular risk factors, and followed for a mean of 12.9 years. Subjects were ranked into quintiles according to baseline cholesterol values, the mean values ranging from 4.8 mmol·L⁻¹ in the lowest quintile to 7.9 mmol·L⁻¹ in the highest, ie, a range of 3.1 mmol·L⁻¹.

| Quintile | Entire cohort (n=21515) | Repeat measurement (n=5696) | | | CHD mortality (entire cohort) |
|---|---|---|---|---|---|
| | Mean baseline cholesterol | Mean repeat cholesterol | Δ Cholesterol | LDL cholesterol | Age-adjusted rate/1000 man years |
| **1** | 4.8 | 5.0 | +0.2 | 3.3 | 1.11 |
| **2** | 5.6 | 5.6 | 0 | 3.9 | 1.57 |
| **3** | 6.2 | 6.1 | -0.1 | 4.3 | 1.86 |
| **4** | 6.8 | 6.5 | -0.3 | 4.7 | 2.01 |
| **5** | 7.9 | 7.2 | -0.7 | 5.3 | 3.11 |
| **Range quintiles 1-5** | **3.1** | **2.2** | **-** | **2.0** | **2.0** |

**Table III.** *Effect of repeated measurement of total and low-density lipoprotein (LDL) cholesterol on gradient of coronary heart disease/cholesterol relationship in the BUPA Study. Data from Law et al,[15] see text for discussion.*

Repeat measurements of total and LDL cholesterol were made in 5696 men after an average of 3 years. The mean baseline values in each quintile of this subgroup were identical to those of the equivalent quintile of the whole cohort, yet the spread of mean values on repeat measurement was reduced to 5.0 to 7.2 mmol·L^{-1} (range 2.2 mmol·L^{-1}) as a result of regression to the mean. Thus the near threefold difference in CHD mortality between quintiles 1 and 5 corresponded to a difference of 3.1 mmol·L^{-1} in initial cholesterol, but only 2.2 mmol·L^{-1} in repeat cholesterol. The estimated difference in CHD mortality per 0.6 mmol·L^{-1} difference in cholesterol is therefore increased from 17% for initial cholesterol to 24% after correction for regression dilution bias.

## SURROGATE DILUTION EFFECT

A further issue in the interpretation of epidemiological data is that the majority of studies have used baseline measurements of total cholesterol, yet it is LDL cholesterol that is most closely linked to CHD risk. *Table III* illustrates this point, by indicating that the span of LDL cholesterol values between the highest and lowest risk quintiles in the BUPA study was 2.0 mmol·L^{-1}, as opposed to a range of 2.2 mmol·L^{-1} in total cholesterol. There was no significant difference in high-density lipoprotein (HDL) cholesterol or in very-low-density lipoprotein (VLDL) cholesterol between quintiles 1 and 5, therefore the total cholesterol values may be used as an acceptable surrogate for LDL levels. However, the steepness of the association between total cholesterol and risk underestimates the true relationship between LDL cholesterol and risk. In the study by Law et al,[15] the estimate of reduction in relative risk per 0.6 mmol·L^{-1} reduction in

total cholesterol was increased from 17% to 24% by correction for regression dilution bias and further to 27% by allowance for surrogate dilution bias. Use of these correction factors in the review of previous epidemiological studies led to the overall estimate of 27% per 0.6 mmol·L^{-1} cholesterol reduction in the cohort studies and 38% in the international studies.[6]

## REGRESSION AND SURROGATE DILUTION EFFECTS IN THE STATIN TRIALS

What relevance do regression and surrogate dilution effects have for the interpretation of the cholesterol lowering trials? If the treatment effect is calculated from the difference between the mean values at baseline and on-treatment, the accuracy of the estimate is dependent on the validity of these two measurements. The baseline cholesterol value represents the result either of a single measurement[1,3-5] or the mean of two levels taken 4 weeks apart.[2] As such, these values do not represent the "usual" levels for the individual, which can only be determined over a longer period of observation. The baseline values in the trials are therefore not corrected for regression dilution bias.

In contrast, the on-treatment values in the statin trials were derived from the average of multiple measurements taken during follow-up, except in AF/TexCAPS, where a single measurement at 12 months was reported. Thus the on-trial measurements are likely to be freer from regression dilution bias, but since the mean reduction in cholesterol is calculated as the difference between the baseline value and the on-trial value, the inaccuracy in the former will

compromise the validity of the estimate. The apparently steeper cholesterol reduction/risk reduction relationship in the AF/TexCAPS than in the other trials *(Table II)* may also be explained by the greater imprecision in both the baseline and on-treatment values of cholesterol in this study. The issue of surrogate dilution bias does not affect the estimates of risk reduction/LDL reduction given in *Table II.* However, the ratio of Δ total cholesterol/ΔLDL in the trials ranged from 1.0 to 1.08, an average value of 1.06, as opposed to the figure of 1.14 observed in the BUPA study, and used as the correction factor in the meta-analysis of the international and cohort studies discussed above. Use of this lower figure would reduce the expected risk reduction per 0.6 mmol·L^{-1} cholesterol reduction in the BUPA study from 27% to 25%.

## OVERESTIMATION BIAS?

The discussion thus far has assumed that the figures for risk reduction in the epidemiological studies represent the "true" association between cholesterol and CHD risk. On this basis, the results of the statin trials appear to fall short of the predicted effect. An alternative explanation should be considered, namely, that the epidemiological studies might overestimate the strength of the association. This possibility could arise if the differences in mean cholesterol levels between populations in international studies were associated with other, unmeasured, differences in risk profile. Although the studies quoted by Law et al attributed 80% of the international variation in CHD mortality to cholesterol, recent data from the MONICA study (MONItoring of trends and determinants in CArdiovascular disorders–

World Health Organization)[16] have identified threefold differences in CHD incidence between Belfast, Northern Ireland, and Toulouse, France, despite almost identical mean levels of cholesterol, blood pressure, and smoking. Clearly, other risk factors may play a role, and recent attention has focussed on antioxidant vitamin intake, plasma fibrinogen levels, and the role of chronic infection with agents such as *Chlamydia pneumoniae* or *Helicobacter pylori.* Unrecognized differences in risk factors may well explain why the difference in CHD risk per 0.6 mmol·L^{-1} change in total cholesterol is greater in international studies than in cohort studies (38% vs 27%) in the overview by Law et al.[6] Even in cohort studies, the possibility of differences in unrecognized risk factors, which are correlated with cholesterol, cannot be excluded. These might amplify the gradient of the CHD risk/cholesterol association.

In contrast, the philosophy of randomized clinical trial design recognizes that not all differences in risk profile between the active and placebo groups can be corrected statistically, since not all risk factors are known. The only way to minimize differences in unmeasured risk factors between two groups is to use large sample sizes with random allocation of treatment. The intervention in the statin trials is specific, involving inhibition of the enzyme HMG-CoA reductase, which results in significant reductions in plasma total and LDL cholesterol, with minor reductions in triglycerides and increases in HDL cholesterol. The drugs have minor effects on plasma fibrinogen, but no other known effects on reduction in CHD risk. The effects of the drugs on unrecognized risk factors for CHD are, of course, unknown.

## COMPLIANCE

Cholesterol-lowering therapy is most effective in patients who take medication regularly over a long period. There was a 29% to 30% withdrawal rate from statin therapy in the primary prevention[2,4] and 6% to 19% in the secondary prevention studies.[1,3,5] These withdrawals may represent only the tip of a larger iceberg of poor compliance. Although more than 95% of subjects still receiving medication appeared to be compliant by tablet counts, this is an inaccurate method of assessment. No reliable technique exists to determine long-term compliance with statin therapy, and since the drugs lower cholesterol within 2 to 4 weeks, even the lipid levels taken at follow-up visits may be unrepresentative, since subject compliance may improve in the period prior to a scheduled trial visit.

The impact of compliance was assessed by the WOSCOPS investigators,[17] who demonstrated that when analysis was restricted to subjects who attended and were issued with study medication at ≥75% of visits, the reduction in risk of nonfatal myocardial infarction or CHD death increased from 31% to 38%. This increased the estimated risk reduction per 0.6 mmol·L^{-1} cholesterol reduction from 13% to 16%.

## EFFECT OF TRIAL DURATION

The development of coronary atherosclerosis takes place over many decades, from the earliest fatty streaks visible in childhood and adolescence. Both cohort studies and international comparisons compare the risk of coronary events to a level of cholesterol present over many decades. In contrast, clinical trials of lipid reduction

have usually lasted not more than 5 years, which may be insufficient time to see the full effect of lipid reduction. The three largest trials of lipid reduction in the pre-statin era showed a "lag" in the onset of clinical benefit of 2 to 3 years,[18-20] after which the event curves in the active and placebo groups began to diverge. In a meta-analysis of all of the pre-statin diet, surgical, and drug trials, Law et al reported that the reduction in CHD risk per 0.6 mmol·L^{-1} reduction in total cholesterol was 7% in the first 2 years since trial entry, 22% between 2.1 to 5 years, and 25% between 5 to 12 years.[6]

Four of the statin trials showed differences in event rates between the placebo and statin groups within the first year,[1,2,4,5] while in the CARE study there was a lag phase of 2 years before a treatment effect was seen.[3] The mean follow-up period in the statin trials has been 5 to 6 years. Thus, the average time to event in the trials is approximately 2.5 to 3 years, at which time the full, epidemiologically predicted effect of lipid reduction would not be expected.

## CONCLUSIONS

The discussion above illustrates the complexity of trying to relate the reduction in CHD risk achieved in the statin trials to the predicted effect. At first sight, lipid lowering with statins appears only to achieve about 50% of the predicted effect. However, when allowance is made for regression dilution bias, poor compliance or noncompliance, and the duration of lipid lowering prior to CHD events in the trials, it is likely that the true benefit of long-term statin therapy will be closer to that predicted from cohort studies.

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# How can we identify patients at high risk of coronary heart disease events?

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*Successful strategies are needed to prevent atherosclerotic plaque development and vessel occlusion and their consequences in terms of cardiac events. "Traditional" risk stratification has used various algorithms based on assessment of standard risk factors and the characteristics and extent of atherosclerotic cardiovascular disease in order to match the intensity of intervention to the level of risk at an acceptable cost. Recent studies have focused on the importance of novel prognostic factors and cardiac markers to predict the risk of future events, including inflammation (C-reactive protein), thrombosis (fibrinogen), direct assessment of the extent of disease (cardiac imaging techniques), predictors of the vulnerable plaque (serum troponin T), as well as cultural, social, and behavioral predictors of outcome such as depression and poor social support.*

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Given the dramatic progress made in understanding the genesis and consequences of atherosclerotic plaque development and vessel occlusion, it is not surprising that multiple potentially beneficial therapies have been developed to prevent the consequences of plaque progression and rupture. However, as with most medical therapies, our portfolio of treatments is not curative. We have succeeded in developing effective strategies for delaying the progression of the disease and for ameliorating some of its consequences, but not for absolutely preventing it. Furthermore, these approaches to prevention and treatment come at a cost. Given the global nature of the disease and its enormous consequences,[1] we cannot afford to use every possible medical approach. Rather, we must attempt to define the risk to patients over time and then apply the level of intervention that is appropriate to the level of risk.

## RISK STRATIFICATION

Before discussing risk factors, it is important to define what is being predicted by the risk factors. This issue may be considered from the point of view of pathophysiology or clinical outcome. We would like to be able to identify patients at risk of progression of underlying atherosclerosis, of a plaque event (disruption of the plaque causing unstable angina or myocardial necrosis), of adverse remodeling of the left ventricle causing heart failure, or of sudden ventricular dysrhythmia. While a construct for how these events occur is necessary for devising new risk factors, in the practical application of risk prediction we must keep in mind the primary patient desires: living longer, feeling better, avoiding unpleasant events, and spending less money. From this perspective the major events to predict are death, myocardial infarction, heart failure, sudden death, and symptomatic angina, which are the clinical consequences of the pathophysiologic processes mentioned above. While much progress has been made in predicting clinical events, our efforts to predict quality of life in its multiple dimensions are rudimentary. This perspective on clinical events and quality of life also emphasizes the importance of identifying patients at risk who can be treated preemptively to avoid the cost of expensive procedures such as revascularization.

The etiology of atherosclerosis is complex, and epidemiological studies have been unable to identify a single "smoking gun" that can predict risk. Rather, the risk of cardiac events appears to be multifactorial, reflecting multiple ways to reach the same end of a symptomatic cardiac event as a consequence of dynamic alterations of atherosclerotic plaque, the neurohormonal environment, the propensity for thrombosis,

and inflammation. Because of this complexity, we cannot know in advance if a pair of risk factors will be additive or multiplicative (synergistic), or whether they are simply providing redundant information about risk. Empirical observations are necessary to sort out these complex relationships.

Critical to the modern understanding of risk stratification is the concept of matching the intensity of intervention to the level of risk. In general, effective therapies exert a proportional reduction in the risk of events so that, for the same risk reduction, the therapy will prevent more events per treated patient

in higher-risk patients. Thus, bypass surgery has greater absolute benefit in patients with more severe coronary heart disease (CHD), statins save more lives per 100 patients treated in patients with high low-density lipoprotein (LDL) cholesterol, and angiotensin-converting (ACE) inhibitors save
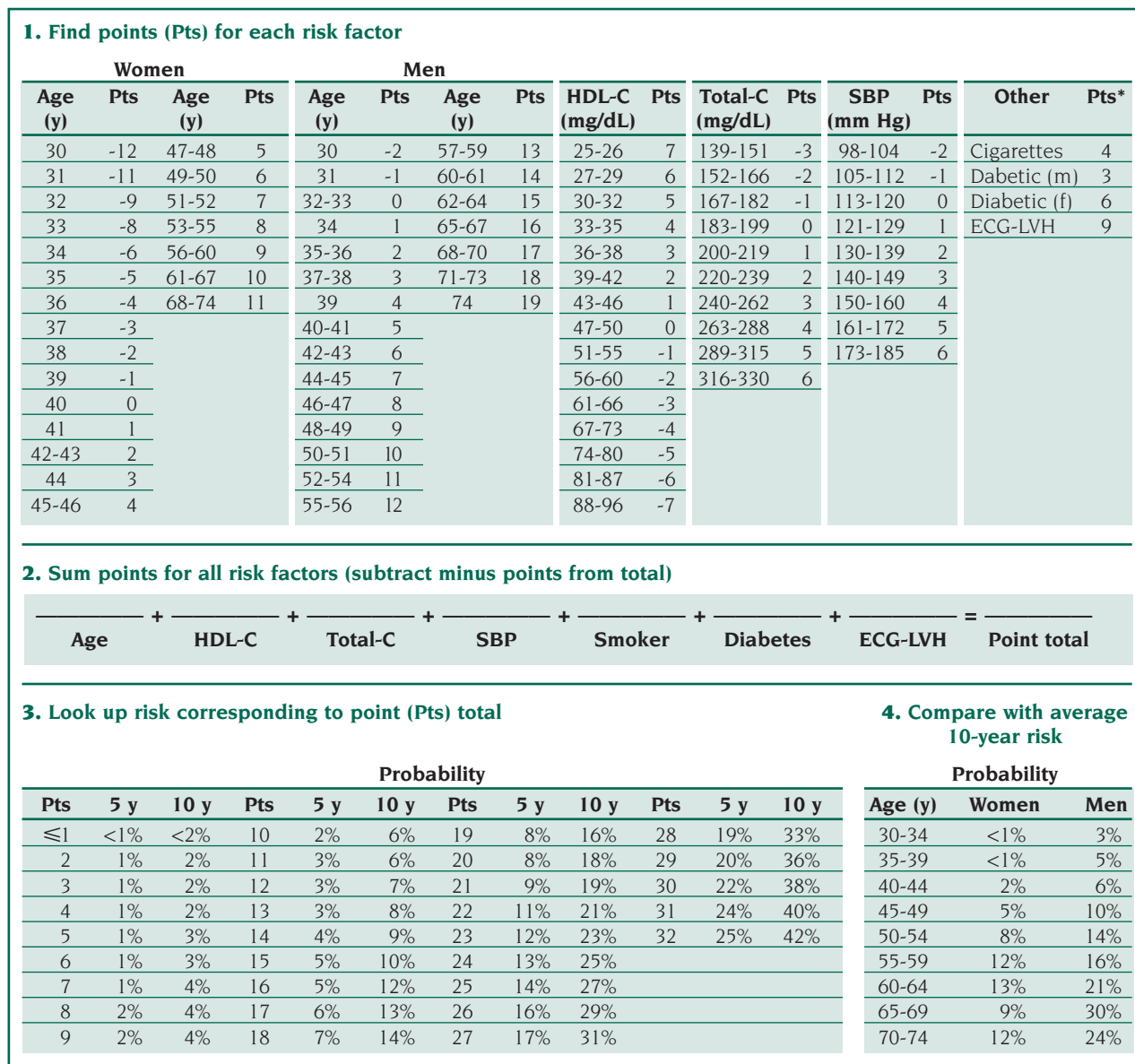
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### 1. Find points (Pts) for each risk factor

| | Women | | | | Men | | | HDL-C (mg/dL) | Pts | Total-C (mg/dL) | Pts | SBP (mm Hg) | Pts | Other | Pts* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age (y) | Pts | Age (y) | Pts | Age (y) | Pts | Age (y) | Pts | | | | | | | | |
| 30 | -12 | 47-48 | 5 | 30 | -2 | 57-59 | 13 | 25-26 | 7 | 139-151 | -3 | 98-104 | -2 | Cigarettes | 4 |
| 31 | -11 | 49-50 | 6 | 31 | -1 | 60-61 | 14 | 27-29 | 6 | 152-166 | -2 | 105-112 | -1 | Dabetic (m) | 3 |
| 32 | -9 | 51-52 | 7 | 32-33 | 0 | 62-64 | 15 | 30-32 | 5 | 167-182 | -1 | 113-120 | 0 | Diabetic (f) | 6 |
| 33 | -8 | 53-55 | 8 | 34 | 1 | 65-67 | 16 | 33-35 | 4 | 183-199 | 0 | 121-129 | 1 | ECG-LVH | 9 |
| 34 | -6 | 56-60 | 9 | 35-36 | 2 | 68-70 | 17 | 36-38 | 3 | 200-219 | 1 | 130-139 | 2 | | |
| 35 | -5 | 61-67 | 10 | 37-38 | 3 | 71-73 | 18 | 39-42 | 2 | 220-239 | 2 | 140-149 | 3 | | |
| 36 | -4 | 68-74 | 11 | 39 | 4 | 74 | 19 | 43-46 | 1 | 240-262 | 3 | 150-160 | 4 | | |
| 37 | -3 | | | 40-41 | 5 | | | 47-50 | 0 | 263-288 | 4 | 161-172 | 5 | | |
| 38 | -2 | | | 42-43 | 6 | | | 51-55 | -1 | 289-315 | 5 | 173-185 | 6 | | |
| 39 | -1 | | | 44-45 | 7 | | | 56-60 | -2 | 316-330 | 6 | | | | |
| 40 | 0 | | | 46-47 | 8 | | | 61-66 | -3 | | | | | | |
| 41 | 1 | | | 48-49 | 9 | | | 67-73 | -4 | | | | | | |
| 42-43 | 2 | | | 50-51 | 10 | | | 74-80 | -5 | | | | | | |
| 44 | 3 | | | 52-54 | 11 | | | 81-87 | -6 | | | | | | |
| 45-46 | 4 | | | 55-56 | 12 | | | 88-96 | -7 | | | | | | |

### 2. Sum points for all risk factors (subtract minus points from total)

—————— + —————— + —————— + —————— + —————— + —————— + —————— = ——————
  Age        HDL-C        Total-C        SBP        Smoker        Diabetes        ECG-LVH        Point total

### 3. Look up risk corresponding to point (Pts) total

### 4. Compare with average 10-year risk

| | Probability | | | | | | | | | | | | Probability | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pts | 5 y | 10 y | Pts | 5 y | 10 y | Pts | 5 y | 10 y | Pts | 5 y | 10 y | Age (y) | Women | Men |
| ≤1 | <1% | <2% | 10 | 2% | 6% | 19 | 8% | 16% | 28 | 19% | 33% | 30-34 | <1% | 3% |
| 2 | 1% | 2% | 11 | 3% | 6% | 20 | 8% | 18% | 29 | 20% | 36% | 35-39 | <1% | 5% |
| 3 | 1% | 2% | 12 | 3% | 7% | 21 | 9% | 19% | 30 | 22% | 38% | 40-44 | 2% | 6% |
| 4 | 1% | 2% | 13 | 3% | 8% | 22 | 11% | 21% | 31 | 24% | 40% | 45-49 | 5% | 10% |
| 5 | 1% | 3% | 14 | 4% | 9% | 23 | 12% | 23% | 32 | 25% | 42% | 50-54 | 8% | 14% |
| 6 | 1% | 3% | 15 | 5% | 10% | 24 | 13% | 25% | | | | 55-59 | 12% | 16% |
| 7 | 1% | 4% | 16 | 5% | 12% | 25 | 14% | 27% | | | | 60-64 | 13% | 21% |
| 8 | 2% | 4% | 17 | 6% | 13% | 26 | 16% | 29% | | | | 65-69 | 9% | 30% |
| 9 | 2% | 4% | 18 | 7% | 14% | 27 | 17% | 31% | | | | 70-74 | 12% | 24% |

**Figure 1.** *Coronary heart disease risk factor prediction chart: patients without known coronary artery disease.*

*Abbreviations and symbols: *, zero points for each "No"; ECG-LVH, electrocardiographic left ventricular hypertrophy; f, female; HDL-C, high-density lipoprotein cholesterol; m, male; Pts, points; SBP, systolic blood pressure; Total-C, total cholesterol.*

more lives in patients with more depressed left ventricular function. Thus, in addition to informing patients about what to expect, a critical goal is to identify patients in whom the benefit of intervening exceeds the risk of intervening at an acceptable cost.

Defining risk of atherosclerotic cardiovascular disease can be considered in several dimensions: stage of disease, time within the stage of disease, and type of risk factor. The quantification of risk must begin with simple measurements that can be made in the course of a routine encounter, but more expensive measures must be used when they can target patients with the most to gain from intervention. Patients who have already experienced a manifestation of atherosclerosis are at much higher risk in general than those with no apparent clinical manifestation of the disease. In defining the risk of individuals in these categories, risk factors may be divided into "traditional" risk factors and novel risk factors, which are in the process of being defined.

## "TRADITIONAL" RISK FACTORS

The most useful information about defining risk in individuals without a previous manifestation of CHD comes from the Framingham Heart Study. Over the course of many years, the Framingham investigators have measured multiple risk factors and quantified their relationship to clinical events during follow-up. *Figure 1* depicts the "classic" Framingham risk model.[2] By knowing the age, sex, high-density lipoprotein (HDL) cholesterol, total cholesterol, systolic blood pressure, smoking status, diabetes status, and whether left ventricular hypertrophy is present on the electrocardiogram, a prediction can be made for the individual person about the risk of a future cardiac event. These equations have been applied in a variety of populations, and they predict outcomes effectively regardless of the culture or geographic location. However, only a portion of the attributable risk is defined by these risk factors, and much more needs to be known.

In patients with documented CHD, the risk of future events can be more precisely defined, and much of cardiovascular clinical practice has been dedicated to using technology to estimate this risk. The risk assessment begins with the standard risk factors enumerated above. Unfortunately, once a patient has been identified as having the disease, these "traditional" risk factors are much less important than the extent of the disease at that time point. Once a CHD event has occurred, the patient may be considered to be in one of several disease states: stable CHD, acute coronary syndrome with or without ST-segment elevation, post–coronary revascularization, or heart failure/left ventricular dysfunction. Each of these disease states has its own particular characteristics associated with specific risks of poor outcomes.

Substantial work, as demonstrated in *Figure 2*,[2] has been done to elucidate the prognosis of patients with chronic, stable CHD. The traditional risk factors add only



**New MI/unstable score:**

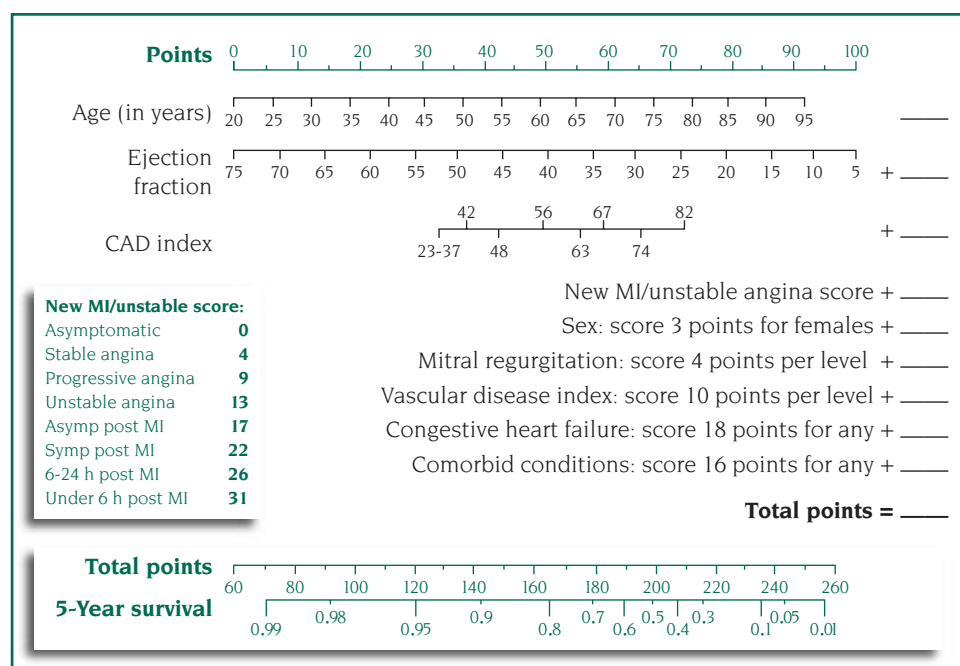| | |
|---|---|
| Asymptomatic | 0 |
| Stable angina | 4 |
| Progressive angina | 9 |
| Unstable angina | 13 |
| Asymp post MI | 17 |
| Symp post MI | 22 |
| 6-24 h post MI | 26 |
| Under 6 h post MI | 31 |

*Figure 2.* Nomogram for prediction of 1-year survival based on clinical, physical examination, and cardiac catheterization findings.

Abbreviations: Asymp, asymptomatic; CAD, coronary artery disease; MI, myocardial infarction; Symp, symptomatic.

Reproduced with permission from ref 2: Califf RM, Armstrong PW, Carver JR, D'Agostino RB, Strauss WE. 27th Bethesda Conference: matching the intensity of risk factor management with the hazard for coronary disease events. Task Force 5. Stratification of patients into high, medium and low risk subgroups for purposes of risk factor management. J Am Coll Cardiol. 1996;27:1007-1019. Copyright © 1996, American College of Cardiology.

a modest amount to risk when other aspects of the disease are well characterized. The history is important for characterizing evidence of previous myocardial infarction, severity of angina, and severity of heart failure symptoms, if any. The recent history of the severity and tempo of angina is particularly important in the patient without previous left ventricular dysfunction. Evidence of peripheral vascular disease or cerebral vascular disease also adds to the risk. Of course, the physical examination provides relatively limited information in the typical patient with CHD, but detection of signs of heart failure, valvular dysfunction, or diffuse atherosclerosis can be valuable. Diagnostic and prognostic testing also adds to the prediction of risk. Measures of left ventricular function, provocative tests for ischemia, and measures of exercise capacity provide information that is only partially redundant with information obtained from the history and physical examination. Finally, coronary angiography provides critical prognostic information, albeit at a relatively high cost and risk.

A particularly interesting and large population consists of persons with subclinical atherosclerosis. Because these individuals far outnumber the population with clinically evident atherosclerosis, the opportunity is much greater for intervening effectively to make a major difference. However, because the absolute event rate is low, the cost of intervention for the amount of benefit to the individual may be prohibitive unless effective risk stratification methods are developed.

When patients have an acute plaque event, they may remain asymptomatic (probably the case in most events) or they may develop an

acute coronary syndrome (ACS). When the "culprit" vessel does not occlude, and large platelet emboli do not occur, the symptomatic patient typically has non–ST-segment-elevation ACS. In the presence of vessel occlusion or large platelet emboli, the patient either has non–ST-segment-elevation ACS with positive markers of myocardial necrosis or ST-segment-elevation ACS. Risk stratification should begin in these patients from the moment of first evaluation. Thus, the risk stratification algorithms have much in common, but differences are also apparent.

Patients with non–ST-segment-elevation ACS are a heterogeneous population. The specific configuration of the electrocardiogram is important to estimate their risk.[3] A detailed analysis of the Platelet glycoprotein IIb/IIIa in Unstable angina: Receptor Suppression Using Integrelin Therapy (PURSUIT) data identified a variety of demographic and clinical features available upon admission to the hospital that are important in stratifying risk. Age is a critical factor, while findings reflecting the hemodynamic state (blood pressure, heart rate, rales), whether or not infarction was present, and measures of the extent of atherosclerosis dominate the risk assessment. Additional information is now available indicating that markers of myocardial necrosis (troponin I and troponin T) can add substantially to the clinical information,[4-7] leading to the routine use of these markers in the assessment of patients in the emergency department.

Extensive risk stratification has been done in patients with ST-segment-elevation ACS. The key factors are age, the hemodynamic state of the patient, the extent of myocardial necrosis, the extent

of atherosclerosis, and the degree of electrical instability. Lee and colleagues have developed a risk stratification model based on over 40 000 patients in the Global Use of Strategies To Open occluded coronary arteries (GUSTO)-I trial,[8] and this model has been extended to include a detailed evaluation of the electrocardiogram.[9] A nomogram depicting these elements of risk is displayed in *Figure 3*.[9]

The assessment of risk in a patient with ACS should continue throughout the hospitalization as depicted in *Figure 4 (page 144)*.[10] The patient with ACS has an increased risk of death that persists for several months after diagnosis; after that time the risk reverts to that of the patient with chronic, stable angina.

Patients with CHD and heart failure are subject to the same risk factors as patients with chronic CHD without heart failure but, in addition, the specific characterization of the heart failure adds important information. This information may be considered in the categories of severity of symptoms of heart failure, measures of exercise capacity,[11] and measures of neurohormonal activation.

Once a patient has experienced a symptomatic ventricular arrhythmia or a resuscitated sudden death event, it intuitively makes sense that a new set of prognostic features would be of value. While ambulatory monitoring, signal-averaged electrocardiography, and heart rate variability measures have been found to provide prognostic information, these measures have not been demonstrated to be useful in selecting patients for intervention. Given the efficacy and the cost of the implantable cardioverter/defibrillator, better risk stratification measures are urgently needed.
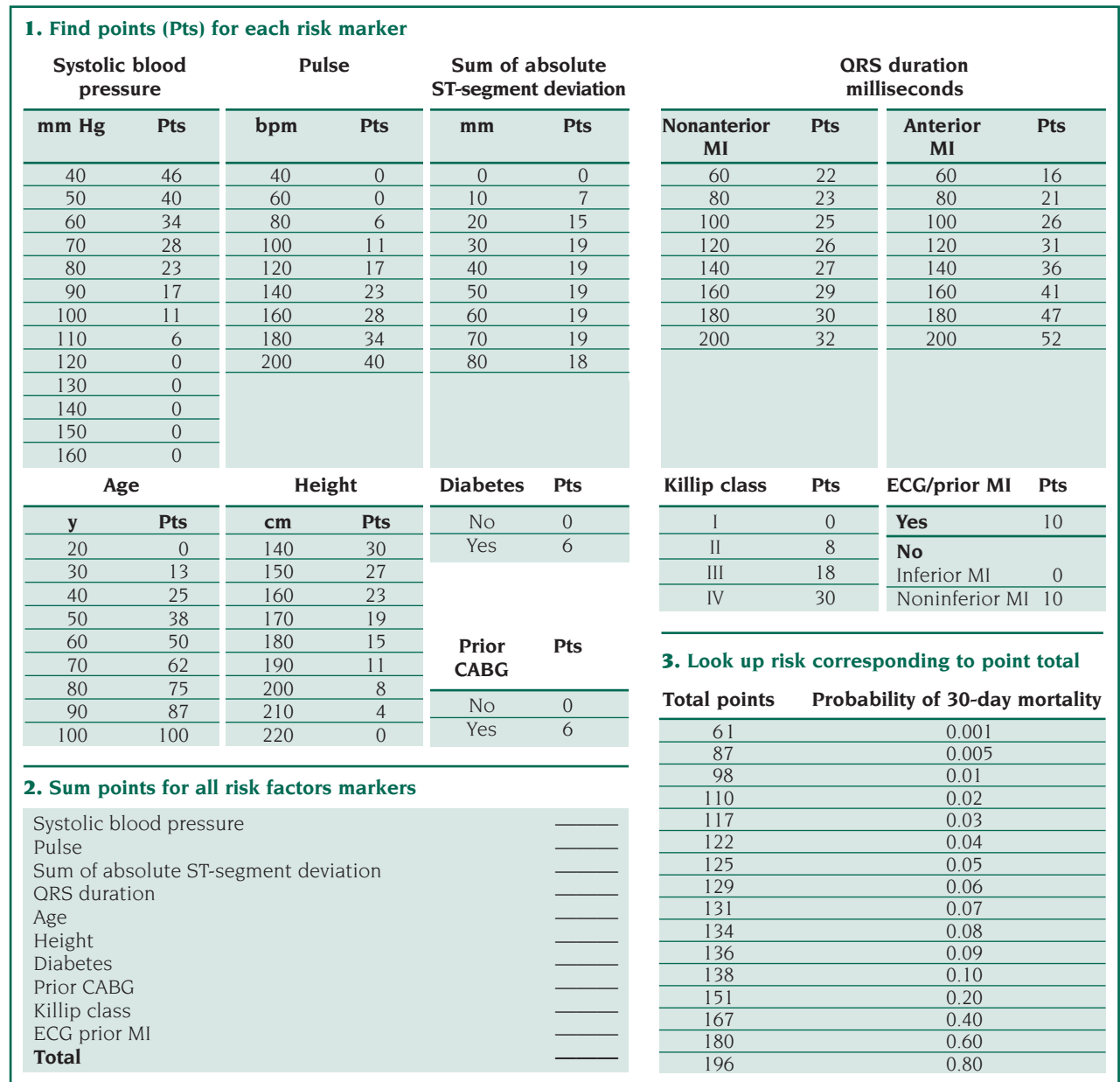
## 1. Find points (Pts) for each risk marker

| Systolic blood pressure | | Pulse | | Sum of absolute ST-segment deviation | |
|---|---|---|---|---|---|
| **mm Hg** | **Pts** | **bpm** | **Pts** | **mm** | **Pts** |
| 40 | 46 | 40 | 0 | 0 | 0 |
| 50 | 40 | 60 | 0 | 10 | 7 |
| 60 | 34 | 80 | 6 | 20 | 15 |
| 70 | 28 | 100 | 11 | 30 | 19 |
| 80 | 23 | 120 | 17 | 40 | 19 |
| 90 | 17 | 140 | 23 | 50 | 19 |
| 100 | 11 | 160 | 28 | 60 | 19 |
| 110 | 6 | 180 | 34 | 70 | 19 |
| 120 | 0 | 200 | 40 | 80 | 18 |
| 130 | 0 | | | | |
| 140 | 0 | | | | |
| 150 | 0 | | | | |
| 160 | 0 | | | | |

| QRS duration milliseconds | | | |
|---|---|---|---|
| **Nonanterior MI** | **Pts** | **Anterior MI** | **Pts** |
| 60 | 22 | 60 | 16 |
| 80 | 23 | 80 | 21 |
| 100 | 25 | 100 | 26 |
| 120 | 26 | 120 | 31 |
| 140 | 27 | 140 | 36 |
| 160 | 29 | 160 | 41 |
| 180 | 30 | 180 | 47 |
| 200 | 32 | 200 | 52 |

| Age | | Height | | Diabetes | Pts |
|---|---|---|---|---|---|
| **y** | **Pts** | **cm** | **Pts** | No | 0 |
| 20 | 0 | 140 | 30 | Yes | 6 |
| 30 | 13 | 150 | 27 | | |
| 40 | 25 | 160 | 23 | | |
| 50 | 38 | 170 | 19 | | |
| 60 | 50 | 180 | 15 | **Prior CABG** | **Pts** |
| 70 | 62 | 190 | 11 | | |
| 80 | 75 | 200 | 8 | No | 0 |
| 90 | 87 | 210 | 4 | Yes | 6 |
| 100 | 100 | 220 | 0 | | |

| Killip class | Pts | ECG/prior MI | Pts |
|---|---|---|---|
| I | 0 | **Yes** | 10 |
| II | 8 | **No** | |
| III | 18 | Inferior MI | 0 |
| IV | 30 | Noninferior MI | 10 |

## 2. Sum points for all risk factors markers

| | |
|---|---|
| Systolic blood pressure | ——— |
| Pulse | ——— |
| Sum of absolute ST-segment deviation | ——— |
| QRS duration | ——— |
| Age | ——— |
| Height | ——— |
| Diabetes | ——— |
| Prior CABG | ——— |
| Killip class | ——— |
| ECG prior MI | ——— |
| **Total** | ——— |

## 3. Look up risk corresponding to point total

| Total points | Probability of 30-day mortality |
|---|---|
| 61 | 0.001 |
| 87 | 0.005 |
| 98 | 0.01 |
| 110 | 0.02 |
| 117 | 0.03 |
| 122 | 0.04 |
| 125 | 0.05 |
| 129 | 0.06 |
| 131 | 0.07 |
| 134 | 0.08 |
| 136 | 0.09 |
| 138 | 0.10 |
| 151 | 0.20 |
| 167 | 0.40 |
| 180 | 0.60 |
| 196 | 0.80 |

***Figure 3.*** *Nomogram for estimating 30-day mortality from initial clinical and electrocardiographic variables. This reduced version of the full multivariable model yielded a C-index statistic of 0.830.*

*Abbreviations: bpm, beats/min; MI, myocardial infarction; ECG, electrocardiogram; CABG, coronary artery bypass graft.*

*Reproduced with permission from ref 9: Hathaway WR, Peterson ED, Wagner GS, et al, for the GUSTO-I Investigators. Prognostic significance of the initial electrocardiogram in patients with acute myocardial infarction.* JAMA. *1998;279:387-391. Copyright © 1998, American Medical Association.*

## NOVEL PROGNOSTIC FACTORS

Recent studies have focused on the importance of markers of inflammation in predicting the risk of future events. Initial studies looked at patients with acute coronary syndromes and found a concentration of events in those with elevated levels of C-reactive protein, a general marker of inflammation.[12] These early observations were followed by long-term observations in the chronic setting[13] and evaluations of other markers of inflammation,[14] all leading to the same conclusion: inflammation is a major issue in atherosclerosis. Still unsolved is the question of whether the inflammation is a causative factor or a result of the atherosclerotic process.

Since the consequences of plaque disruption are largely mediated by
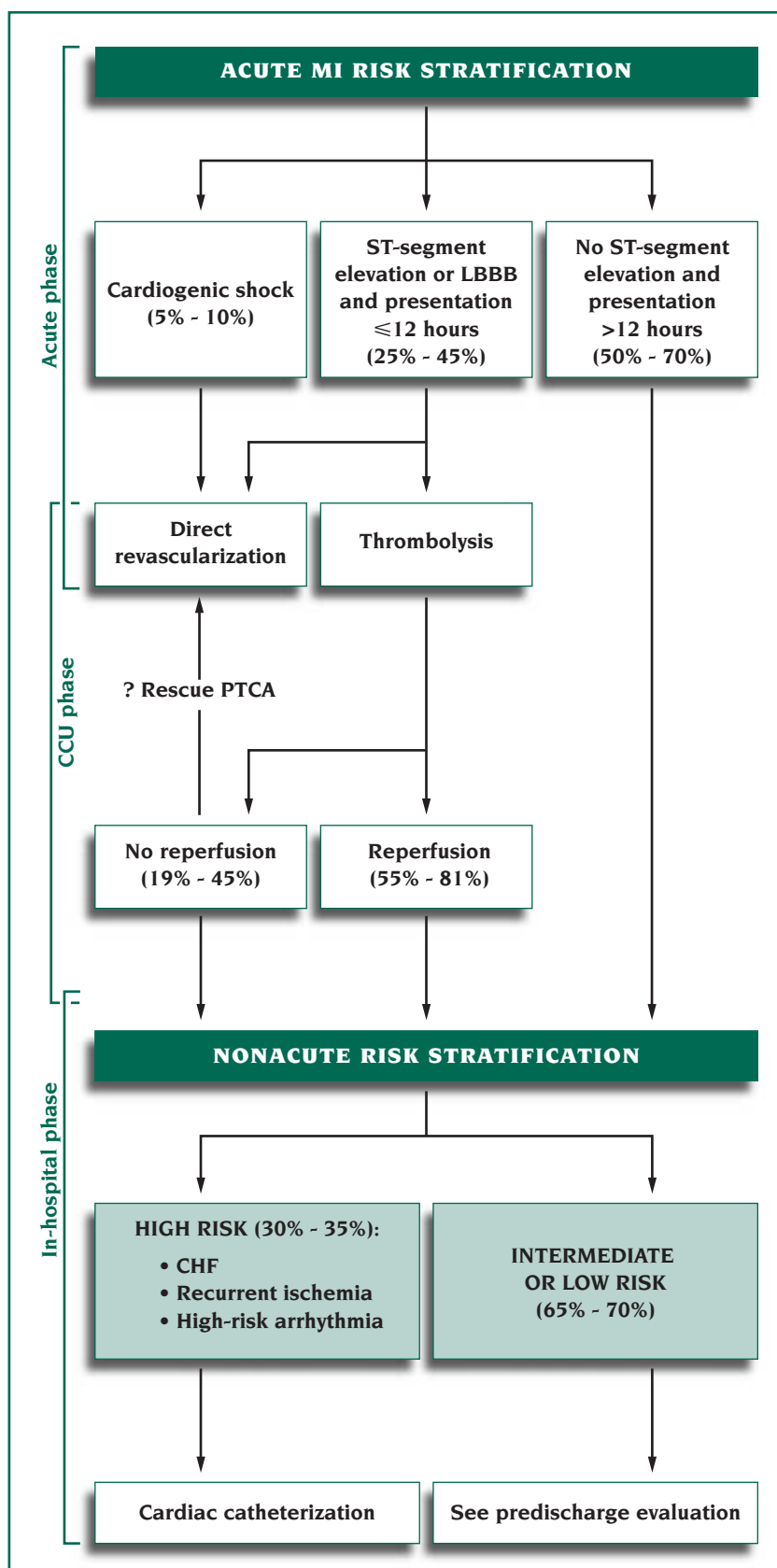
the degree to which thrombotic occlusion obstructs the vessel lumen, a logical conclusion is that elevated markers of thrombosis should predict which patients are likely to have events. Despite the demonstration that there is a relationship between outcome and thrombotic markers, the relationships have not been strong enough to incorporate measuring these markers to stratify risk into clinical practice. Fibrinogen has emerged as a major predictor across a large number of studies; it may become the first thrombotic marker to be used in routine risk prediction.

The field of cardiac imaging is progressing at a rapid pace, bringing about the possibility of directly measuring the extent of disease and following it as a marker of prognosis. Rapid electron beam computed tomography (CT) scanning has developed as a method of detecting and following coronary calcification in a semiquantitative fashion.[15] Similarly, positron emission tomography (PET) provides vivid semiquantitative information about ischemia and viability. While these technologies have great promise, they have not yet accumulated enough evidence to determine their place in clinical practice.

We also cannot forget about cultural, social, and behavioral issues in risk stratification. Recent studies



***Figure 4.*** *Flow diagram of risk stratification after myocardial infarction.*

*Abbreviations: CCU, coronary care unit; CHF, congestive heart failure; LBBB, left bundle-branch block; MI, myocardial infarction; PTCA, percutaneous transluminal coronary angioplasty; ST, ST-segment.*

*Reproduced with permission from ref 10: Peterson ED, Shaw LJ, Califf RM. Clinical Guideline: Part II. Risk stratification after myocardial infarction. Ann Intern Med. 1997;126:561-582. Copyright © 1997, American College of Physicians - American Society of Internal Medicine.*

have implicated depression[16,17] and poor social support[18] as major predictors of poor outcome. Whether these differences in outcome result from physiological manifestations of the underlying disorders or whether depression and social isolation lead to lower compliance and less medical care remains to be resolved.

Perhaps the most intriguing target for risk prediction is the vulnerable plaque. Most of our efforts to predict the risk of events are limited because angiographic and physiologic measures characterize the state of the arteries, the myocardium, or the hemodynamic state in a static situation. Yet we know that a person with normal left ventricular function, normal neurhormones, and no ischemia on provocative testing can be transformed into a victim of acute myocardial infarction almost instantaneously when a vulnerable plaque becomes disrupted. The troponins may be giving us insight into the degree to which a "hot" plaque is shedding platelet emboli and markers of inflammation may provide indirect insight into weakening of the plaque by inflammatory infiltrates, but we do not yet have direct measures that can discern a vulnerable plaque.

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# How can we stratify patients with unstable angina and determine optimal therapy?

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*Acute coronary ischemia results from pathophysiological processes that include coronary atherosclerosis, plaque rupture, and coronary thrombosis. Unstable angina is the most common acute coronary syndrome. However, its diagnosis may be unclear initially, and even in patients who are eventually shown to have this diagnosis, risk can vary widely. In addition, a diverse array of investigations and therapeutic options is available. These facts underline the importance of accurate risk stratification, with the aim of appropriate management, whether medical therapies or, in addition, early invasive investigation and possibly revascularization. In this article, early risk stratification, the impact of measurement of troponins, the appropriate timing of coronary angiography, and role of medical therapies, particularly newer pharmacological classes—glycoprotein IIb/IIIa inhibitors and low-molecular-weight heparins—are discussed.*

**Keywords:** unstable angina; coronary thrombosis; risk stratification; antiplatelet therapy; antithrombotic therapy; coronary angiography

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## DIAGNOSIS AND "CONVENTIONAL" RISK STRATIFICATION

Acute coronary syndromes manifest as unstable angina or non–Q-wave or Q-wave myocardial infarction. It is common experience that, of them, unstable angina is the most frequent.[1] A number of possible presentations of unstable angina are recognized:

- Angina at rest (typically, lasting for more than 20 minutes).

- New onset (ie, within 2 months) exertional angina, resulting in marked or severe limitation of physical activity.

| HIGH RISK | INTERMEDIATE RISK | LOW RISK |
|---|---|---|
| *At least one of the following present* | *No high-risk feature, but any of the following* | *Any of the following may be present* |
| • Prolonged (>20 min) ongoing rest angina | • Prolonged rest angina, now resolved, with moderate or high likelihood of coronary artery disease | • Increased angina frequency, severity, or duration |
| • Pulmonary edema, most likely related to ischemia | • Rest angina | • Angina provoked at a lower threshold than previously |
| • Angina at rest with dynamic ST-segment changes >1 mm | • Nocturnal angina | • New-onset angina with onset 2 weeks to 2 months before presentation |
| • Angina with new or worsening mitral regurgitation | • Angina with dynamic T-wave changes on the ECG | • Normal or unchanged ECG |
| • Angina, with third heart sound or new/worsening pulmonary rales | • New-onset, moderate angina in past 2 weeks with moderate likelihood of coronary artery disease | • Younger age |
| • Angina with hypotension | • Pathological Q waves or resting ST-segment depression >1 mm in multiple leads on the ECG | |
| • Other important features<br>- Diabetes<br>- Older age | • Age >65 years | |

***Table I.** Short-term risk of death or nonfatal myocardial infarction with unstable angina.*

• Recent (ie, within 2 months) acceleration of angina by at least one Canadian Cardiovascular Society Class (CCSC) to at least CCSC class III.[2]

In addition, variant angina, non–Q-wave myocardial infarction, and postmyocardial infarction angina (ie, more than 24 hours after the event) are often included in the diagnosis of unstable angina.

In usual practice, the initial assessment of patients is based on information obtained from clinical history, physical examination, and the 12-lead electrocardiogram (ECG). The two objectives are to define the likelihood of significant coronary heart disease (CHD)—which depends on assessment of the patient's history, past history of CHD, age of the patient, and hemodynamic or ECG changes—and risk of adverse short-term outcomes.

A classification based on the nature of the presentation was prepared by Braunwald in 1989.[3] This was prospectively validated by Calvin et al[4] who found that, in addition, advanced age and diabetes portended a poorer outcome. *Table I* shows a practical risk classification adapted and simplified from this work, of the type which might until recently have been applied in "conventional" practice.

## NEW ASPECTS OF EMERGENCY ASSESSMENT

Two particular developments, assay for troponin I and troponin T, and the use of "chest pain clinics" for rapid triage and assessment after hospital presentation may improve the risk stratification and allow more appropriate management. Around 10% to 20% of all patients presenting with unstable angina are at low risk in the short term,

| SELECTED ABBREVIATIONS AND ACRONYMS | |
|---|---|
| **APTT** | activated partial thromboplastin time |
| **CAPTURE** | Chimeric 7E3 AntiPlateleT in Unstable angina REfractory to standard treatment trial |
| **CCSC** | Canadian Cardiovascular Society Class (of angina) |
| **EPIC** | Evaluation of IIb/IIIa platelet receptor antagonist 7E3 in Preventing Ischemic Complications study |
| **ESSENCE** | Efficacy and Safety of Subcutaneous Enoxaparin in non–Q-wave in Coronary Events study |
| **FRIC** | FRagmin In unstable Coronary artery disease study |
| **FRISC** | FRagmin during InStability in Coronary artery disease study |
| **GUSTO** | Global Use of Strategies To Open occluded coronary arteries studies |
| **LIPID** | Long-term Intervention with Pravastatin in Ischemic Disease study |
| **OASIS-2** | Organizations to Assess Strategies for Ischemic Syndromes–2 trial |
| **PARAGON** | Platelet IIb/IIIa Antagonist for the Reduction of Acute coronary syndrome events in a Global Organization Network trial |
| **PRISM** | Platelet Receptor inhibition for Ischemic Syndrome Management study |
| **PRISM-PLUS** | Platelet Receptor inhibition for Ischemic Syndrome Management in Patients Limited by Unstable Signs and Symptoms |
| **PTCA** | percutaneous transluminal coronary angioplasty |
| **PURSUIT** | Platelet IIb/IIIa Underpinning the Receptor for Suppression of Unstable Ischemia Trial |
| **TIMI** | Thrombin Inhibition in Myocardial Ischemia studies |
| **TRIM** | Thrombin Inhibition in Myocardial Infarction trial |
| **VANQWISH** | Veterans Affairs Non–Q-Wave Infarction Strategies in Hospital study |

with a 30-day death or myocardial infarction rate of <1%, while up to 55% of all patients have an intermediate course with a 6% event rate at 30 days.[5] Thus, a significant proportion of patients may be managed with shorter hospital admissions and early outpatient investigations rather than prolonged inpatient admissions.

The troponins are highly sensitive and specific markers of myocardial cell necrosis. A number of studies have demonstrated the value of troponin T assays in the prediction of cardiac events. Hamm et al studied a population of 109 consecutive patients admitted with unstable angina, most of whom had angina at rest during the previous 24 hours.[6] The positive predictive value of troponin T for prediction of in-hospital death or myocardial infarction was 30%. Its negative predictive value was 98%. Other reported studies have shown that elevated troponin T remains predictive of death and myocardial infarction at 34 months.[7,8] Data from the Fragmin during INstability in Coronary artery disease (FRISC) study[9] demonstrated increasing risk with increasing troponin T levels. In a population mostly composed of patients with new angina, there was a gradual increase in death and myocardial infarction with increasing troponin T levels. After 5 months' follow-up of those presenting with unstable angina, the risk of cardiac death and myocardial infarction for troponin levels <0.06 µg/L, 0.06-0.18 µg/L, and >0.18 µg/L was 4.4%, 11.4%, and 14.1%, respectively. The risk of this combined end point in those presenting with non–Q-wave myocardial infarction was 17.7%.

In a further study by Hamm et al, in 773 consecutive patients presenting with acute chest pain and no new ST-segment changes on ECG, those with normal troponin levels were discharged from the emergency department.[10] In patients without elevation in troponin, the 30-day event rates for death or myocardial infarction was 1.1% for troponin T and 0.3% for troponin I. This is concordant with other data suggesting that the lack of elevation in troponin I or T may identify a population of patients in whom the risk of death

and myocardial infarction is low, and a policy of early discharge safe.

In addition to new sensitive and specific markers of myocardial necrosis, the development of chest pain clinics may allow more appropriate selection of patients who benefit from hospital admission. This was investigated in a community-based study by Farkouh et al.[11] Patients with acute coronary syndromes thought to be at intermediate risk were observed for 24 hours with serial measurements of serum creatine kinase. A functional test for cardiac ischemia was performed the following morning if creatine kinase levels were not elevated. Those with negative functional tests were discharged from hospital, and those with positive results were admitted. There was no increase in adverse cardiac events (death, myocardial infarction and congestive cardiac failure) at follow-up at 30 days or at 6 months. As in other studies, most of the observed events occurred in the first few weeks after presentation and assessment. Importantly, the mean duration of stay in the "chest pain clinic" was 9.2 hours, and although there may have been an increase in representation in the group treated via the "clinic" (8.0% vs 4.2%, NS), there was a significant overall reduction in the need for admission to hospital. From the perspective of cost/efficiency, it is unclear whether the routine use of cardiac nuclear studies or stress echocardiography will diminish the cost/benefits of reduced hospital stay.

### Recommendations

*Clear clinical features identify patients at high risk. A history of rest angina within the prior 48 hours, especially associated with prior stable angina, which suggests more extensive underlying*

*coronary artery disease, and the occurrence of ST-segment deviation with pain, especially despite the institution of appropriate medical therapy, defines a group of patients with significant 30-day rates of myocardial infarction and mortality. The age of the patient and the presence of diabetes are also important. In addition, evidence of hemodynamic compromise such as the development of cardiac failure or mitral incompetence with pain suggests that these patients will benefit significantly from admission to a cardiac intensive care area as well as early coronary angiography and possible revascularization.*

*The evidence at hand also suggests that, when available, the use of troponin assays can contribute significantly to risk stratification and should be performed, primarily in the low and intermediate risk populations, where the absence of other clinical and ECG features is an insensitive means of defining those safe for early discharge. The question as to whether assay for troponin T or troponin I is preferable remains to be completely resolved, although most published data relate to troponin T. Troponin T has greater sensitivity, but lesser specificity than troponin I, which is undetectable in healthy persons.*

*Intermediate-risk and low-risk patients constitute the majority presenting with acute coronary syndromes, most of whom will not require admission to intensive monitoring areas. These patients lack the features of high-risk patients, have an excellent 30-day cardiovascular event rate, and may be managed in general wards or on an outpatient basis. The use of chest pain clinics and early functional testing as well as sensitive*

*tests for myocardial necrosis may be able to identify patients presenting with unstable angina who may be safely managed on an outpatient basis, without the need for hospital admission.*

*The improved ability to stratify risk in patients with acute coronary syndromes has important implications for the design and allocation of resources. The shifting paradigm of acute coronary syndromes away from acute myocardial infarction requires a reconsideration of the nature of coronary care units and cardiac intensive care units. Increased utilization of lower dependency areas, with less invasive monitoring, reduced nursing levels, but increased patient education, together with the use of chest pain clinics, may result in more efficient use of health care resources.*

## THE ROLE OF CORONARY ANGIOGRAPHY AND REVASCULARIZATION

Despite its frequent widespread use in clinical practice in many hospitals, there is a lack of clear randomized controlled data to support "routine" use of early coronary angiography in the treatment of patients with unstable angina and non–Q-wave myocardial infarction  Advocates of an approach of early angiography often base this on data that show an advantage of bypass surgery in particular situations. For example, the Veterans Administration Cooperative Study[12] randomized 468 patients. Those with unstable angina and three-vessel disease showed a significant survival benefit for early surgical therapy over continued medical therapy at 5 years (89% vs 75%, $P$<0.02) with most of the benefit seen in those with poor left ventricular function.

A strategy of early invasive therapy for unstable angina and non–Q-myocardial infarction with angiography at 18 to 24 hours and revascularization was assessed in 1473 patients in the TIMI IIIB study.[13] Patients randomized to a strategy of conservative management underwent angiography and revascularization for "failed" medical therapy (especially ongoing symptoms). There was no difference in the primary end point of death, myocardial infarction, and unsatisfactory symptom-limited exercise test at 6 weeks with a conservative approach compared to elective angiography within 48 hours with revascularization when suitable coronary anatomy was demonstrated. However, the group managed conservatively had lower rates of myocardial infarction and death, but at the cost of longer initial hospitalization time and greater need for rehospitalization. Twelve-month follow-up again showed no difference between the two strategies, though revascularization was more frequent in those having early angiography, with a higher rate of coronary angioplasty. Rates of coronary bypass surgery were similar. By 12 months, the difference in percutaneous transluminal coronary angioplasty (PTCA) rates had diminished with an increase in procedural rate in the group who was initially treated conservatively.

These results were echoed in the Veterans Affairs Non–Q-Wave Infarction Strategies in Hospital (VANQWISH) trial comparing early invasive management of patients with non–Q-wave myocardial infarction with a conservative strategy of angiography and revascularization for symptoms or reversible ischemia found on functional testing.[14] Again, the strategy of early angiography was associated with a greater rate of revascularization and increas-

ed hospital stay, but was associated with increased mortality and myocardial infarction, though the differences diminished over 12 months.

However, although the weight of evidence favors an initial conservative approach, more recent unpublished data from the FRISC-II study (presented at the American College of Cardiology meeting, New Orleans, 1999) support a benefit of coronary angiography at some time after presentation. This is consistent with the pragmatic approach of many cardiologists.

### Recommendations

*The published data indicate that "elective" angiography and subsequent revascularization when suitable anatomical findings are demonstrated does not decrease the rate of myocardial infarction and death during the first year of follow-up. This justifies a usual strategy of conservative management with deferral of coronary angiography. Invasive investigation should be utilized for patients who have evidence of ongoing ischemia, despite medical management or demonstrable reversible abnormalities on noninvasive nuclear or echocardiographic imaging. However, the full published results of the FRISC-II study must be awaited.*

## CURRENT MEDICAL THERAPY AND LIMITATIONS

The management of unstable angina has evolved from therapies aimed at the metabolic imbalance of the ischemic myocardium. However, few randomized prospective trials have documented the intermediate and long-term impact of β-blockers, calcium antagonists, and nitrates,

although most have demonstrated effective symptom relief.[15]

More recent approaches have been directed particularly towards the critical role of thrombosis in acute coronary syndromes, in which platelet adhesion, activation, and aggregation are fundamental. The mainstays of antiplatelet and antithrombotic therapies have been aspirin and intravenous heparin. Aspirin irreversibly inhibits cyclooxygenase, preventing affected platelets from synthesizing thromboxane $A_2$, a potent vasoconstrictor and stimulator of platelet aggregation. Its efficacy was documented in the 1980s. A representative trial, the Veterans Administration Cooperative study, randomized 1266 men to aspirin or placebo and found a 50% reduction in fatal and nonfatal myocardial infarction at 12 weeks.[16] A very large meta-analysis in over 90 000 patients supports the use of aspirin in acute coronary syndromes for prevention of vascular events.[17]

More recently, the use of clopidogrel, an ADP-receptor antagonist, was compared with aspirin in a placebo-controlled trial of 19 185 patients as secondary prevention of vascular events. There was a small, statistically significant, but not very clinically significant, reduction in stroke, myocardial infarction, or vascular death in favor of clopidogrel. With a similar tolerability, and similar clinical efficacy at greater cost, clopidogrel is a useful alternative in those unable to tolerate aspirin. Whether aspirin adversely effects angiotensin-converting enzyme efficacy at a clinical level, due to renal and vascular prostaglandin inhibition, should be evaluated in prospective trials before this concept can be used as justification for the use of clopidogrel in preference to aspirin.

Heparin has been the prototype anticoagulant used in patients with unstable angina. It binds with antithrombin, enhancing its ability to inactivate factor Xa and thrombin. With regard to intravenous heparin therapy, Theroux et al randomized 479 patients to aspirin, heparin, or both, for a duration of 6±3 days.[18] Both therapies individually, as well as their combination, were superior to placebo in reducing refractory ischemia, myocardial infarction, or death, though the study was not sufficiently powered to detect a difference between the treatment groups. Importantly, Theroux et al subsequently reported the reactivation of events of refractory angina, myocardial infarction, and death, after the cessation of heparin in the same population of patients.[19] At a mean of 9 hours after the cessation of heparin, the benefit of adding heparin to aspirin was lost. This phenomenon is thought to be due to the presence of residual thrombus as well as the consumption of antithrombin III by prolonged heparin infusions.

Oler et al published a meta-analysis of 6 randomized trials that assessed the addition of unfractionated heparin to aspirin for the treatment of unstable angina in 1353 patients.[20] There was a trend towards reduced death and myocardial infarction (risk ratio, 0.67; 95% confidence interval [CI], 0.44-1.02; $P$=0.06) in the groups receiving heparin at the end of the studies, all of which were less than 1 week. However, only 4 studies reported results to 12 weeks, in a total of 1141 patients, and much of the trend in benefit was attenuated (risk ratio, 0.82; 95% CI, 0.56-1.20; $P$=0.76).

A number of factors may contribute to the limited efficacy of unfractionated heparin and reactivation of acute ischemia. The need for antithrombin,

with reduced amounts or availability of this molecule in the context of active thrombosis, combined with the reduced effectiveness of the heparin-antithrombin complex in the presence of fibrin monomers that protect thrombin from inactivation, may have a role. The release of platelet factor 4 in response to heparin may also contribute to reactivation.

Other limitations of unfractionated heparin are the need for continuous infusions, preventing outpatient management, and difficulty in achieving target activated partial thromboplastin times, given a wide variation in antithrombotic response. Heparin-induced thrombocytopenia also occurs at a rate of around 1% to 3%.[21]

Although it has not been definitively established that the combination of heparin and aspirin is superior to heparin alone, guidelines usually recommend their combined use in patients with unstable angina or non–Q-wave myocardial infarction who are considered to be at moderate-to-high risk.

### NEW THERAPIES FOR UNSTABLE ANGINA
#### Low-molecular-weight heparins

Low-molecular-weight-heparins are derived from unfractionated heparin by depolymerization. The different preparations have different saccharide chain lengths. Low-molecular-weight heparins have greater activity against factor Xa than thrombin, and greater bioavailability with less binding to plasma proteins and endothelial cells than unfractionated heparin. This allows the practical advantage of a more predictable antithrombotic dose-response relationship without the need for laboratory monitoring.

| Trial | LMWH | n | Deaths/MI/Refractory UAP (Death/MI for FRISC) | | |
|---|---|---|---|---|---|
| | | | LMWH (%) | Placebo (%) | *P* |
| FRISC | Dalteparin vs placebo, 6 D | 1506 | 1.8 | 4.8 | 0.001 |
| | Dalteparin vs placebo, 40 D | 1493 | 8.0 | 10.7 | 0.07 |
| FRIC | Dalteparin vs UF Hep, 6 D | 1482 | 9.3 | 7.6 | 0.33 |
| | Dalteparin vs placebo, 6-45 D | 1132 | 12.3 | 12.3 | 0.96 |
| ESSENCE | Enoxaparin vs UF Hep, 2.6 D | 3171 | (14 D) 16.6 (30 D) 19.8 | 19.8 23.3 | 0.02 0.02 |

**Table II.** *Large trials of low-molecular-weight heparins (LMWH) in unstable angina pectoris (UAP)/non–Q-wave myocardial infarction (MI). UF Hep, unfractionated heparin. For meaning of trial acronyms, see box at beginning of article.*

The long half-life of about 4 hours after subcutaneous injections enables twice daily subcutaneous injection. A lower incidence of bleeding has not been confirmed in randomized trials, though a reduced rate of heparin-induced thrombocytopenia has been documented.[21]

The major clinical studies of low-molecular-weight heparin, summarized in *Table II*, have shown low-molecular-weight heparin to be at least as efficacious as, if not superior to, intravenous unfractionated heparin.

FRISC randomized 1506 patients with unstable angina and non–Q-wave infarction to dalteparin or placebo with weight-adjusted twice-daily dosing for six days, followed by single-daily dosing for 35-45 days.[22] At 6 days, there was a significant reduction in death or recurrent myocardial infarction (risk ratio, 0.37; 95% CI, 0.20-0.68) in the dalteparin group, but this effect was diminished at 40 days, though remaining significant for the composite end point of death, myocardial infarction, and revascularization. The FRagmin In unstable Coronary artery disease (FRIC) study randomized 1482 patients with unstable angina and non–Q-wave myocardial infarction, to either a weight-adjusted twice-daily dalteparin dose or activated

partial thromboplastin time (APTT)–adjusted heparin infusion for 6 days.[23] This was followed by a single daily subcutaneous dose of dalteparin or placebo for 6 to 45 days. During both phases of the study, there was no statistically significant difference between the groups with regard to death, myocardial infarction, recurrent angina, and revascularization. Because of its size and rigorous design, the Efficacy and Safety of Subcutaneous Enoxaparin in non–Q-wave Coronary Events (ESSENCE) study provides the best available evidence that a low-molecular-weight heparin is superior to unfractionated heparin.[24] This study randomized patients to weight-adjusted enoxaparin twice daily or APTT-adjusted intravenous heparin infusions. Duration of therapy was between 48 hours and 8 days. At 30 days, the end point of death, myocardial infarction, and recurrent angina was 19.8% vs 23.3% in favor of the enoxaparin limb, with a similar rate of major bleeding.

## Recommendations

*The role of low-molecular-weight heparin in the treatment of acute coronary syndromes is expanding. Benefits of dalteparin have been demonstrated in placebo-controlled trials, and superiority established*

*for enoxaparin over unfractionated heparin. Thus, they should be seen as having at least similar (and probably greater) efficacy to standard unfractionated heparin. In addition, the improved dosing predictability eliminates the need for laboratory monitoring, and there is less requirement for intravenous access. These agents also allow greater options for the nursing and management of patients in low-dependency areas or even as outpatients, enabling prolonged anticoagulation before definitive revascularization. Though pharmacologic differences between the low-molecular-weight heparins have been suggested,[25,26] differences in efficacy cannot be directly inferred, due to differences in trial design and the fact that direct comparisons have not been made in formal trials. The possible cost-efficiency of prolonged outpatient low-molecular-weight heparin therapy is very promising, but is yet to be fully defined. However, the data suggest that, clinically, low-molecular-weight heparins should be continued beyond the acute phase in patients awaiting angiography and possible revascularization.*

## Direct antithrombins

Hirudin, hirulog, and argatroban are selective antithrombin agents that

differ from heparin in that they do not require interaction with an intermediate enzyme and bind directly to the catalytic site of thrombin. Proposed benefits over heparin are the ability to inactivate clot-bound thrombin, as well as reduced thrombin-induced platelet activation, reduced factor V and VIII activation, and endothelin release. Antithrombins are not inhibited by platelet factor 4, remain active in platelet-rich environments, and are not associated with thrombocytopenia.

Small studies have suggested a favorable efficacy and safety profile for hirulog in comparison with intravenous unfractionated heparin. The Thrombin Inhibition in Myocardial Ischemia–7 (TIMI-7) study randomized 410 patients to 4 escalating doses of hirulog compared with heparin over 72 hours.[27] When compared to the lowest dose of hirulog, the 3 higher doses did not result in reduction in death, myocardial infarction, or recurrent ischemia in 72 hours, but there was a significant difference in events at hospital discharge and at 6 weeks' follow-up. An angiographic study by Topol et al,[28] again with ranging doses of hirudin, assessed severe coronary stenosis and overt thrombus. Repeat angiography at 72 to 120 hours showed that the 116 patients who received hirudin tended to have greater thrombus resolution and improved angiographic findings. However, large-scale trials of direct antithrombins have shown less benefit.

Most experience in unstable angina is with hirudin. The Global Use of Strategies To Open occluded coronary arteries (GUSTO) IIb study randomized 4131 patients with acute coronary syndromes and ST-segment elevation, and 8011 patients without ST-segment eleva-

tion, to either 72 hours of intravenous heparin or recombinant hirudin.[29] There was a significant reduction in death or myocardial infarction at 24 hours with hirudin (1.3% vs 2.1%, $P$=0.001). However, at 30 days, the benefit was diminished and no longer significant, the risk of death or myocardial infarction being 8.9% in the hirudin group and 9.8% in the heparin group ($P$=0.06). The predominant benefit seen was a reduction in myocardial infarction, and there was no difference between those presenting with ST-segment elevation or depression. There was a higher rate of moderate bleeding complications requiring transfusion in the hirudin group ($P$=0.03). The Organization to Assess Strategies for Ischemic Syndromes–2 (OASIS-2) trial randomized 10 141 patients with unstable angina or suspected acute myocardial infarction without ST-segment elevation to 72-hour infusion of heparin or hirudin.[30] At 7 days, there was a small nonsignificant reduction in cardiovascular death or new myocardial infarction, the primary end point ($P$=0.08); however, there was a significant reduction in refractory angina. The difference persisted to 35 days. When assessed in conjunction with the OASIS-1 data,[31] the preceding pilot study in about 900 patients, there was a reduction in death, myocardial infarction, and refractory angina, although the benefits were small, again at the cost of increased bleeding.

Finally, in the Thrombin Inhibition in Myocardial Infarction (TRIM) trial, inogratan (3 doses) was compared with unfractionated heparin in 1209 patients with unstable angina or non–Q-wave myocardial infarction.[32] The heparin-treated group had significantly fewer ischemic events

within the first 3 days. However, differences were not statistically significant at 7 days (the primary end point) or 30 days.

## Recommendations

*Overall, despite the laboratory and small-scale trial evidence of benefit over standard heparin, only a small incremental benefit has been shown in large-scale studies. The trend in favor of hirudin during on-therapy analysis is attenuated by 30 days, at the cost of increased bleeding and transfusions. Due to low event rates in these large trials, it has been difficult to demonstrate marked differences in therapy, and gains may appear to be small. However, given the varying levels of risk in patients presenting with acute coronary syndromes, there may still be a role for the direct antithrombins in the high-risk populations, where this form of therapy is likely to be more cost-effective.*

## Glycoprotein IIb/IIIa inhibitors

Platelets are at the core of coronary thrombus, owing to their activation and adhesion to exposed subendothelial matrix elements with plaque fissure or rupture. Although platelet aggregation may be stimulated by a variety of substances, considerable evidence suggests that they all operate via a common mechanism, activation of the glycoprotein IIb/IIIa receptor, the platelet membrane receptor for fibrinogen. Antagonists of the glycoprotein IIb/IIIa receptor have been developed as a useful tool in the setting of coronary angioplasty, and have demonstrated efficacy in the setting of unstable angina and non–Q-wave myocardial infarction.

| Trial | Treatment | n | Deaths and MI within 30 days | | |
|-------|-----------|---|-------------------|---|---|
| | | | IIb/IIIa (%) | Control (%) | *P* |
| PRISM | Tirifoban vs heparin | 3231 | 5.8 | 7.1 | 0.11 |
| PRISM PLUS | Tirifoban + heparin vs heparin | 1915 | 8.7 | 11.9 | 0.03 |
| PURSUIT | Eptifibatide + heparin vs placebo + heparin | 10 948 | 14.2 | 15.7 | 0.042 |
| PARAGON A | Lamifoban + heparin vs heparin (+ other regimens) | 2282 | 9.0 | 9.2 | ns |

**Table III.** *Large trials of IIb/IIIa antagonists in unstable angina pectoris (UAP)/non–Q-wave myocardial infarction (MI). For meaning of trial acronyms, see box at beginning of article.*

The first glycoprotein IIb/IIIa antagonist to reach clinical practice was abciximab, a murine chimeric monoclonal Fab antibody fragment, but subsequently both peptides (integrelin), and small nonpeptide molecules (tirofiban, lamifiban, sibrafiban, xemilofiban, and orbofiban) have reached phase III clinical trials. Abciximab has been mainly studied to date in association with percutaneous coronary interventions, (but is being investigated in acute coronary syndromes in GUSTO IV), and there have been no direct comparisons between agents. Major trials are summarized in *Table III*, although care is necessary in any comparisons between these trials because of differences in their design and primary end point.

The Platelet IIb/IIIa Antagonist for the Reduction of Acute coronary syndrome events in a Global Organization Network (PARAGON) trial randomized 2282 patients with unstable angina and new or persistent ECG changes, to low- or high-dose lamifiban in a 2 × 2 factorial study with or without heparin, or to placebo and heparin.[33] At 30 days, the composite end point of death or myocardial infarction had occurred in 11.7% of those receiving standard heparin, and in 10.6% on low-dose lamifiban and 12.0% on high-dose lamifiban, though at 6 months, the composite end point for low-dose lamifiban, high-dose

lamifiban, and standard heparin was 13.7%, 16.4%, and 17.9%, respectively. Low-dose lamifiban was associated with fewer ischemic events and a lower rate of bleeding, while high-dose lamifiban was associated with more bleeding and a similar rate of ischemic events.

Two major trials of intravenous tirofiban have been undertaken in a similar population of patients. The Platelet Receptor inhibition for Ischemic Syndrome Management (PRISM) study randomized 3232 patients with unstable angina or non–Q-wave myocardial infarction documented by either ST-segment–T-wave changes on the ECG and cardiac enzyme changes, or documented prior ischemic heart disease, to tirofiban or heparin for 48 hours.[34] At 48 hours, there was a 32% reduction in death, myocardial infarction, and refractory angina, (risk ratio, 0.67; 95% CI, 0.48-0.92; *P*=0.01), but at 30 days the difference was no longer significant, though a lower mortality was noted. Major bleeding had the same incidence (0.4%) in both groups, and there was a greater incidence of reversible thrombocytopenia in the tirofiban group (1.1% vs 0.4%). The Platelet Receptor inhibition for Ischemic Syndrome Management in Patients Limited by Unstable Signs and Symptoms (PRISM-PLUS) study was limited to patients with ST-segment or T-wave evidence of

acute ischemia and randomized 1915 patients to tirofiban alone, heparin alone, or tirofiban and heparin for 72 h.[35] Due to an increase in the mortality at 7 days, the tirofiban-alone arm was ceased prematurely. However, the composite end point of death, myocardial infarction, or refractory ischemia at 7 days was lower in the group treated with a combination of tirofiban and heparin (12.9% vs 17.9%, *P*=0.004). This difference persisted at 30 days and 6 months, although the benefit was reduced. For the end points of death and myocardial infarction alone the benefit was more prominent, 4.9% vs 8.3% at 7 days, again being maintained to 6 months. Bleeding was similar in the two groups.

In the Platelet IIb/IIIa Underpinning the Receptor for Suppression of Unstable Ischemia (PURSUIT) study, the largest trial to this time, 10948 patients were randomized to intravenous eptifibatide or placebo for 72 to 96 hours, given in addition to aspirin and heparin.[36] At 30 days, there was a small, but significant, reduction in death or myocardial infarction (14.2% vs 15.7%) with eptifibatide (*P*=0.04). There was no increase in stroke, major bleeding, or thrombocytopenia.

The role of the glycoprotein IIb/IIIa inhibitors as adjunctive therapy in the setting of angioplasty for acute

coronary syndromes has been clearly documented. From the Evaluation of IIb/IIIa platelet receptor antagonist 7E3 in Preventing Ischemic Complications (EPIC) study, the subgroup presenting with unstable angina and undergoing angioplasty, 489 patients were randomized to abciximab bolus, bolus and infusion, or placebo.[37] At 30 days, a 62% reduction in the primary end point of death, myocardial infarction, or urgent revascularization was seen in the bolus and infusion group, (4.8% vs 12.8%, *P*=0.012), and the benefit was sustained at 6 months. Most of the benefit was evidenced as reduction in death or myocardial infarction. The Chimeric 7E3 AntiPlateleT in Unstable angina REfractory to standard treatment (CAPTURE) trial also assessed abciximab in patients with refractory unstable angina, unresponsive to standard treatment including heparin and nitrates or placebo, undergoing angioplasty.[38] A total of 1050 patients were given abciximab bolus and infusion or placebo 18 to 24 hours before the planned procedure. The study was terminated early, because of a significant reduction with abciximab in death and myocardial infarction (11.3% vs 15.9%). Again, most of the reduction was in myocardial infarction. Interestingly, there was no longer a difference in composite outcome at 6 months. Also of note was a small benefit already evident in the abciximab limb before intervention. A retrospective analysis of the data suggested that elevated troponin I levels >0.2 units indicated those more likely to derive benefit from abciximab, while those with levels below this showed no statistically significant difference in outcome.

Comparing the specific use of these intravenous agents for different indications, in a large meta-analysis of 16 randomized controlled trials in 32 135 patients, similar benefit was seen when trials were subgrouped by the indication of acute coronary syndrome versus percutaneous intervention.[39] The same overview confirms consistency of benefits in the trials in patients with unstable angina.

Oral glycoprotein IIb/IIIa inhibitors could provide prolonged platelet inhibition and these have potential value in chronic, recurrent ischemia, but thus far have been disappointing. Small studies such as the TIMI-12 trial,[40] a dose-ranging study assessing sibrafiban, have demonstrated effective platelet inhibition with only a minor increase in bleeding. However, recently, a study of sibrafiban was stopped early because of no apparent benefit, and possible increased mortality in the higher-dose limb. Also, no significant benefit has been found in a study of another oral agent, xemilofiban. It may also be necessary to demonstrate that long-term receptor blockade does not lead to upregulation, thus exacerbating ischemia after an oral agent is discontinued.

## Recommendations

*The benefit of the glycoprotein IIb/IIIa inhibitors in the treatment of unstable angina and non–Q-wave infarction is established for the acute phase of the disease, as well as in adjunctive therapy with percutaneous intervention. The benefits of the nonpeptide inhibitors tirofiban, lamifiban, and eptifibatide have been consistent in patients with acute coronary syndromes with early decrease in death, myocardial infarction, and refractory ischemia with or without percutaneous interventions. However, all but the data from the EPIC trial[37] demon-strated that, although maintained, there was some attenuation of benefit at 6 months. The results to date from the trials of oral agents in acute coronary syndromes have been disappointing.*

*Because of their significant cost, the intravenous agents cannot be given to all patients. However, they should be used in high-risk patients. Data from the CAPTURE trial[38] suggest that troponin I may be one tool for identifying high-risk patients who will benefit. Transient ECG changes and ongoing ischemia are also used to identify those patients in whom these agents would be used. Whether defining patients at increased risk by markers of myocardial necrosis and these other clinical observations proves a truly effective means of delineating the role of glycoprotein IIb/IIIa receptor antagonists in clinical practice should be confirmed with prospective clinical trials.*

## OTHER THERAPIES

There is almost certainly undertreatment of recognized coronary risk factors in patients presenting with unstable angina. In particular, in the recently reported Long-term Intervention with Pravastatin in Ischemic Disease (LIPID) study,[41] 3260 patients had unstable angina as the qualifying diagnosis. An average of 6.1 years of treatment with pravastatin resulted in a significant reduction of not only coronary events, but also total mortality compared with placebo-assigned patients.

## CONCLUSIONS

Unstable angina and non–Q-wave myocardial infarction remain a significant and costly public health problem. New approaches to early risk stratification with troponin

and the other rapid markers of myocardial necrosis, as well as the use of specialized triage clinics, will allow for more appropriate allocation of resources, invasive investigation, and effective utilization of expensive therapies. The intravenous glycoprotein IIb/IIIa antagonists reduce cardiovascular events in high-risk patients in the acute phase of the disease as well as after coronary interventions. The low-molecular-weight heparins enable care of intermediate- and low-risk patients to be undertaken in low-dependency areas or on an outpatient basis. Indeed, the low-molecular-weight heparins should perhaps be preferred to unfractionated heparin. Trials with direct thrombin inhibitors have been inconclusive and those with oral glycoprotein IIb/IIIa receptor inhibitors disappointing to date.

*Figure 1* shows a possible management schema incorporating these "newer" approaches. The possible increased risk of bleeding with combinations of these various antithrombotic approaches is noted. The choice between unfractionated and low-molecular-weight heparin in high-risk patients in whom early coronary angiography is planned, say within 12 to 24 hours, is unclear. Ongoing active research will further elucidate the preferred, most cost-efficient management of patients with these important syndromes.
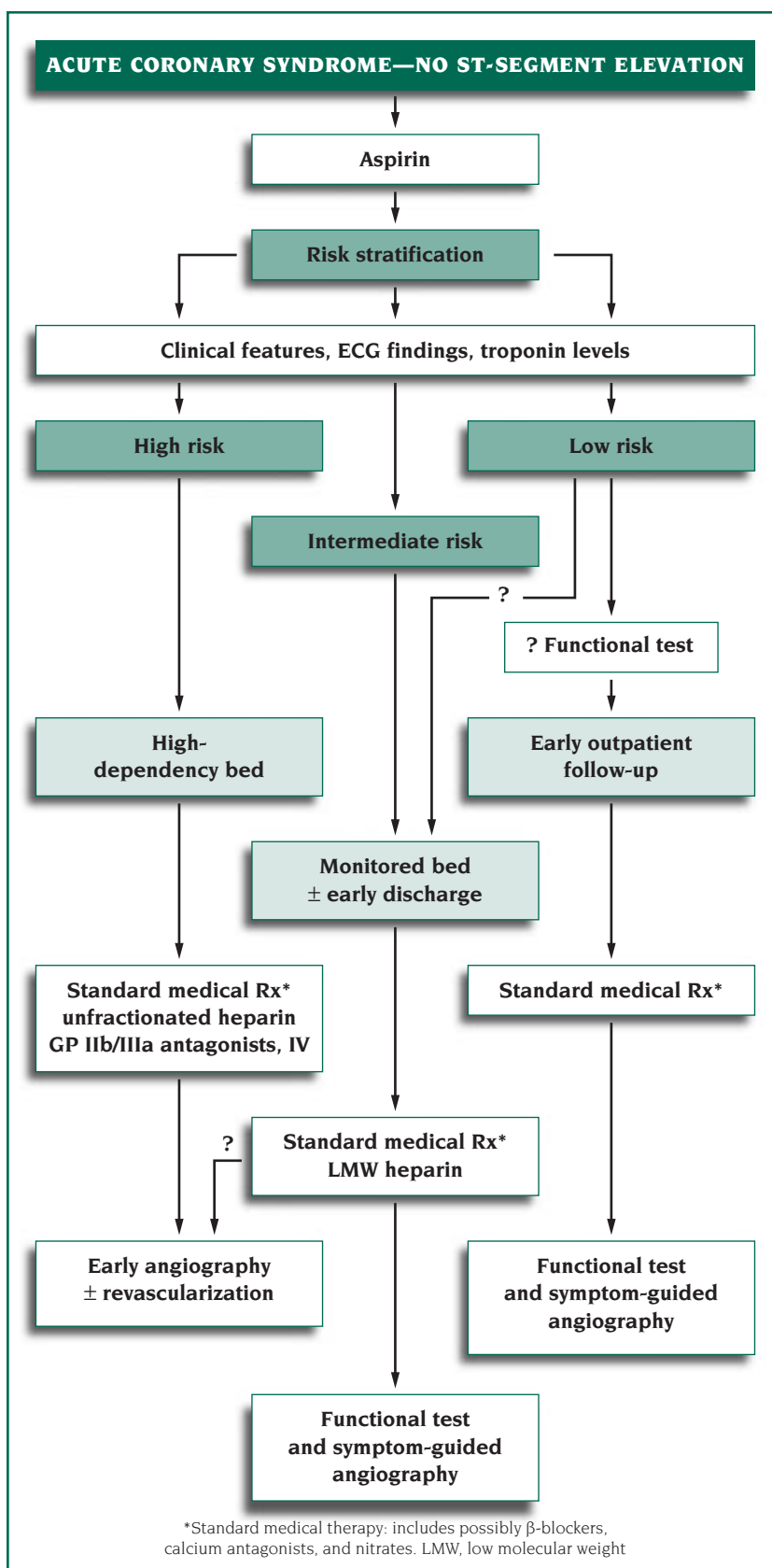


**Figure 1.**
*A management schema incorporating newer approaches.*

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**Circulation.** 1997;96:769-777.

**32. TRIM Study Group.**

*A low-molecular weight, selective thrombin inhibitor, inogatran, vs heparin, in unstable coronary artery disease in 1209 patients.*

**Eur Heart J.** 1997;18:1416-1425.

**33. The PARAGON Investigators.**

*International, randomized, controlled trial of lamifiban (a platelet glycoprotein IIb/IIIa inhibitor), heparin, or both in unstable angina.*

**Circulation.** 1998;97:2386-2395.

**34. The PRISM Study Investigators.**

*A comparison of aspirin plus tirofiban with aspirin plus heparin for unstable angina.*

**N Engl J Med.** 1998; 338: 1498-1505.

**35. The PRISM-PLUS Study Investigators.**

*Inhibition of the platelet glycoprotein IIb/IIIa receptor with tirofiban in unstable angina and non–Q-wave myocardial infarction.*

**N Engl J Med.** 1998;338:1488-1497.

**36. The PURSUIT Trial Investigators.**

*Inhibition of platelet glycoprotein IIb/IIIa with eptifibatide in patients with acute coronary syndromes.*

**N Engl J Med.** 1998;339:436-443.

**37. Lincoff AM, Califf RM, Anderson KM, et al.**

*Evidence of prevention of death and myocardial infarction with platelet membrane glycoprotein IIb/IIIa receptor blockade by abciximab (c7E3) among patients with unstable angina undergoing percutaneous coronary revascularization.*

**J Am Coll Cardiol.** 1997;30:149-156.

**38. The CAPTURE Investigators.**

*Randomised placebo-controlled trial of abciximab before and during coronary intervention in refractory unstable angina: the CAPTURE study.*

**Lancet.** 1997;349:1429-1435.

**39. Kong DF, Califf RM, Miller DP, et al.**

*Clinical outcomes of therapeutic affects that block the platelet glycoprotein IIb/IIIa integrin in ischemic heart disease.*

**Circulation.** 1998;98:2829-2835.

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*Randomized trial of an oral platelet glycoprotein IIb/IIIa antagonist, sibrafiban, in patients after an acute coronary syndrome. Results from the TIMI 12 Trial.*

**Circulation.** 1998;97:340-349.

**41. The LIPID Study Group.**

*The prevention of cardiovascular events and death with pravastatin in patients with coronary heart disease and a broad range of initial cholesterol levels.*

**N Engl J Med.** 1997;339:1349-1357.

# Atheroma

## *Summaries of Ten Seminal Papers*

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Pravastatin has cholesterol-lowering
independent effects on the artery wall
of atherosclerotic monkeys

**J.K. Williams and others.** *J Am Coll Cardiol.* 1998

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British Cardiac Society: ^{125}I-labelled fibrinogen,
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**W.F. Fulton, D.G. Sumner.** *Br Heart J.* 1976

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Unstable angina with fatal outcome: dynamic
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Plaque fissures
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**P. Constantinides.** *J Atheroscler Res.* 1966

Selection of seminal papers by
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# Pravastatin has cholesterol-lowering independent effects on the artery wall of atherosclerotic monkeys

*J.K. Williams, G.K. Sukhova, D.M. Herrington, P. Libby*

One of the most remarkable and consistent effects of cholesterol lowering, whether via β-hydroxy-β-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors or other means, is the dramatic reduction in patient events, with little effect on plaque size. The clinical evidence has therefore strongly suggested that cholesterol lowering has effects on plaque composition, leading to plaque stabilization, irrespective of any effects on plaque regression. The study by Williams et al takes this idea a step forward with the demonstration that small doses of pravastatin can also promote changes in plaque composition that would favor stabilization without lowering serum cholesterol. This strongly argues that pravastatin directly affects plaque cellular biology irrespective of cholesterol lowering.

In this study, the authors fed 32 cynomolgus monkeys an atherogenic diet for 2 years, followed by a lipid-lowering diet for a subsequent 2 years, with or without pravastatin (20 mg/kg body weight/day) at a dose that did not affect total plasma cholesterol or high-density lipoprotein (HDL) cholesterol concentrations. The authors then studied the vasodilator response to acetylcholine (ACh) of the coronary arteries, the plaque size of the coronary arteries by quantitative angiography or that of the iliac arteries by morphometry of removed vessels, and the histology of coronary plaques focusing on calcification, macrophage content, and neovascularization, three characteristics that are all associated with plaque instability. Pravastatin-treated monkeys had preserved vasodilator response to ACh, with fewer macrophages, less neovascularization, and less calcification than non–pravastatin-treated animals. The improvement in function of atherosclerotic arteries and structural composition of plaques occurred despite no change in overall plaque size, or in the percentage of plaques that progressed or regressed angiographically.

Two major questions emerge from this study. First, can pravastatin exert similar effects in humans without the aggressive lipid-lowering diet? Studies of both lovastatin and pravastatin in humans suggest that endothelium-dependent dilatation is improved by these agents, irrespective of lipid lowering. Second, how does pravastatin affect plaque neovessels, calcification, and macrophage numbers in the plaque, and are these effects related? This question has yet to be answered, and a further caveat must be included, notably that plaque rupture/erosion was not studied. The overall effect of pravastatin on plaque stability has therefore not been ascertained. This is important as other factors that determine plaque stability, such as the extent of necrosis within the plaque or fibrous cap size were not affected by pravastatin. It may be that these factors are affected by lipid lowering (indeed, Peter Libby's group has shown that collagen content increases in rabbit vessels after cholesterol lowering by pravastatin), but there is no additional nonlipid effects on cap and necrotic core size of pravastatin in this study. Finally, 20 mg/kg/day is an enormous dose of pravastatin, compared with the doses used clinically. While monkeys are notoriously resistant to cholesterol-lowering drugs, extrapolation of the effects of pravastatin demonstrated in this study to human trials is clearly limited. These caveats aside, this study is the first clear demonstration of beneficial functional and structural effect of HMG-CoA reductase inhibitors independent of lipid-lowering effects. This is likely to be a major direction of future research in this area.

--- 1998 ---

France beats Brazil 3-0 to win the World Cup;
Keiko, the killer whale, star of "Free Willy,"
is returned to the wild;
and "Titanic" sweeps 11 Oscars

# British Cardiac Society: ^{125}I-labelled fibrinogen, autoradiography, and stereoarteriography in identification of coronary thrombotic occlusion in fatal myocardial infarction

*W.F. Fulton, D.G. Sumner*

I n the late 1970s and early 1980s, there was a major paradigm shift in thinking relating to the cause of myocardial infarction. Until that time, the prevalent view had been that coronary artery occlusion occurred due to gradual lumen encroachment of the atherosclerotic plaque, with eventual obliteration of the lumen and vessel closure. In this view, vessel thrombosis was a sequel and not a cause of myocardial infarction. However, pioneering work by Michael Davies and colleagues conclusively demonstrated that acute vessel closure results from superadded blood clot formation onto a ruptured atherosclerotic plaque. The earliest evidence upon which this hypothesis was to be based was the seminal study by Fulton and Sumner, which, although it only appeared in abstract form, sparked a huge interest in this area.

To study thrombosis in patients with myocardial infarction, Fulton and Sumner administered radiolabeled fibrinogen to patients undergoing infarction, and then examined thrombus formation in the artery subtending the infarct zone. If thrombus occurred post infarction, fibrinogen should be incorporated into all thrombus in the affected vessel, as thrombus would have formed after administration of fibrinogen. In contrast, the authors found that although the ends of the occlusive thrombus showed some radiolabel incorporation, the portion of the thrombus causing vessel occlusion was radionegative, suggesting formation before radiofibrinogen administration. This study clearly identifies the timing of thrombosis compared with coronary occlusion, and indicts thrombus formation as the cause and not the consequence of vessel occlusion.

It is hard to overestimate the impact of this paradigm shift. Without this concept, there would be no recognition of the role of plaque stability and rupture as the major determinant of clinical outcome, and no recognition that plaques that rupture are often not flow-limiting. In addition, without the idea that thrombus formation is the cause of occlusion, there is no rationale for the development of thrombolysis for acute myocardial infarction and no scientific basis for the use of antiplatelet agents in unstable angina. A further development is the recognition that a major determinant of survival after myocardial infarction is the speed at which the subtended artery can be reopened and have significant flow. Clearly, a great deal of modern cardiological practice is dependent upon this change in thinking and leap in understanding.

## 1976

Nadia Comaneci, a young Romanian gymnast, wins three gold medals at the Montreal Olympics; Björn Borg, a 20-year-old Swede, wins the Men's Singles at Wimbledon for the first time; and Chairman Mao, leader of the People's Republic of China, dies, aged 82

# Unstable angina with fatal outcome: dynamic coronary thrombosis leading to infarction and/or sudden death. Autopsy evidence of recurrent mural thrombosis with peripheral embolization culminating in total vascular occlusion

*E. Falk*

The seminal observations by Davies and colleagues that myocardial infarction was caused by plaque rupture and subsequent coronary thrombosis, suggested a sudden coronary event causing a defined clinical syndrome. However, this paper by Erling Falk, although it also examined postmortem specimens of acute myocardial infarction/sudden death patients, focused on the generation of the unstable atherosclerotic lesion. Unstable plaques could form by a gradual increase in size over many years, with a sudden rupture leading to acute myocardial infarction. Alternatively, plaques could undergo repeated episodes of minor erosion and rupture followed by healing, with subsequent increase in size of the plaque. This distinction is important, as it means that plaques exist as constantly dynamic structures, eroding and healing, rather than as passive structures that gradually accumulate lipids and inflammatory cells, which then finally rupture, leading to a catastrophic event. Not only would this model predict that plaque growth is episodic, it defines a mechanism by which plaques rapidly increase in size over a few weeks, and indicates a pathophysiological correlation between more minor erosion/rupture and unstable angina.

Falk examined the plaques causing myocardial infarction or sudden death due to coronary occlusion in 25 human cases. Of these, 81% of thrombi had a layered structure with thrombus of various ages. This indicated that such thrombi were formed by progressive mural thrombosis, followed by healing, over an extended period of time, rather than abrupt thrombosis in a single event. Indeed, two or more thrombi were identified in over 81% of cases. These subacute thromboses were clinically silent, although in 73% of cases they were associated with fragmentation of the thrombus with subsequent microembolization and occlusion of small intramyocardial arteries and microinfarcts. In patients with unstable angina before infarction, 14 out of 15 patients showed evidence of repeated thrombus formation and fragmentation. This underscores the idea that unstable angina is caused not by a more severe stenosis than stable angina, but by a more unstable lesion.

The implications of this paper are profound. This was the first demonstration that unstable angina is caused by repeated minor erosions and thrombosis. As such, one would predict that treatment with antiplatelet agents and anticoagulants should reduce the incidence of subsequent myocardial infarction. Numerous clinical trials attest to the validity of this idea. In contrast, antispasmodics (calcium channel blockers, nitrates) or β-blockers have less effect on subsequent myocardial infarction or death, despite reducing the frequency of anginal attacks. If plaques increase in size by repeated episodes of thrombosis, then antiplatelet agents or anticoagulants should reduce the incidence of myocardial infarction in both primary and secondary prevention. Certainly for antiplatelet agents, the data for this effect are good. If plaques are dynamic structures, then clinically silent plaques can still be rapidly progressing before a catastrophic rupture with coronary occlusion. The stabilization (or "passivation") of asymptomatic plaques may be the major mechanism of action of β-hydroxy-β-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors in both primary and secondary prevention trials.

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## 1985

Halley's comet reappears in the skies
for the first time in 75 years;
"Amadeus" wins the Best Picture Oscar;
and Hollywood star Rock Hudson dies, aged 59

# Decreased lesion formation in CCR2-/- mice reveals a role for chemokines in the initiation of atherosclerosis

*L. Boring, J. Gosling, M. Cleary, I.F. Charo*

**Nature.** 1998;394:894-897

Macrophages are important cells in the pathogenesis of atherosclerosis, with macrophages being evident in fatty streaks, one of the earliest lesions of atherosclerosis. Monocytes migrate from the circulating blood to form tissue macrophages, where they oxidize low-density lipoprotein (LDL) and accumulate lipid. Macrophages release a huge array of both chemotactic and mitogenic factors, which contribute to cellular migration and proliferation, promoting plaque formation. However, the signals that initiate monocyte/macrophage recruitment to the vessel wall have only recently been studied. Chemokines are potent proinflammatory cytokines that cause leukocyte chemoattraction in many disease states. In addition, the chemokine monocyte chemoattractant protein (MCP)–1 is upregulated in human atherosclerosis, in animal models of atherosclerosis, and in vascular smooth muscle cells exposed to oxidized lipids, suggesting a causal role for this chemokine in monocyte recruitment in early atherosclerosis.

To study the role of MCP-1 in atherogenesis, Boring et al generated chemokine receptor 2 (CCR2, the receptor for MCP-1) knockout mice, and crossed them with mice lacking apolipoprotein E, which develop spontaneous atherosclerosis or rapidly progressive atherosclerosis if fed a cholesterol-rich diet. Macrophages were abundant in atherosclerotic lesions in control ApoE -/- animals, but much reduced in animals that also lacked CCR2. Lesion size, extent of aorta covered with atherosclerosis, and lipid accumulation in lesions were also reduced in the absence of CCR2, indicating that this receptor is critically important for monocyte recruitment to the vessel wall and lipid accumulation. Heterozygotes for CCR2 showed an intermediate level of atherosclerosis, indicating a dose effect of CCR2. In addition, lesions in CCR2 -/- animals were less complex than in controls, indicating that chemokine receptors are critical in the progression of atherosclerosis, and thus in the development of complications of atherosclerosis. These effects of CCR2 ablation were independent of any change in serum total cholesterol or triglycerides or changes in lipid profiles in CCR2-/-animals, indicating that they were due to non–cholesterol-mediated effects on monocyte recruitment.

The fundamental role of monocyte/macrophages in atherogenesis has been emphasized by similar studies to this, showing that atherosclerosis is severely impaired in monocytes lacking scavenger receptors or in animals lacking MCP-1. Given the increasing number of chemokines and chemokine receptors, it is remarkable that individual chemokines and receptors play such a fundamental, nonredundant role in atherogenesis. Clearly, monocytes/macrophages exert a plethora of effects in atherogenesis, but blocking either their recruitment or lipid accumulation is sufficient to inhibit atherosclerosis. These studies should be taken against the prevailing view that vascular smooth muscle cells were the major guilty cell type in atherosclerosis. In contrast, studies such as this one by Boring et al demonstrate conclusively that macrophages are the major cell type that both initiates and promotes atherosclerosis, thus identifying new targets for prevention and treatment.

## 1998

"Posh Spice" Victoria Adams announces her engagement to footballer David Beckham; "Scary Spice" Mel C. marries dancer Jimmy Gulzar; "Ginger Spice" Geri Halliwell sings "Happy Birthday" to Prince Charles

# Compensatory enlargement of human atherosclerotic coronary arteries

*S. Glagov, E. Weisenberg, C.K. Zarins, R. Stankunavicius, G.J. Kolettis*

Atherosclerosis is an intimal disease, with accumulation of cells and lipid occurring in the intima. In contrast, the arterial media and adventitia had been thought to play minimal roles in atherosclerosis. The study by Glagov et al demonstrated that, far from being passive bystanders in atherosclerosis, the media and adventitia are capable of dynamic remodeling to compensate for the expanding atherosclerotic intima, ameliorating the stenotic effects of atherosclerosis.

Glagov et al studied 136 human hearts at postmortem, examining the extent of atherosclerosis and vessel size (determined by the area circumscribed by the internal elastic lamina [IEL]) of the left main stem. The IEL area correlated directly with the area of the atherosclerotic lesion, indicating that vessels enlarge as the atherosclerotic plaque expands. Indeed, lesion size was a more important predictor of vessel size than either heart weight or age, indicating that arterial dilatation with age may reflect increasing atherosclerosis. This compensatory vessel dilatation means that atherosclerotic lesions can be accommodated in human vessels without a reduction in lumen size. Indeed, when lesion area was compared with percent stenosis (defined as lesion area/IEL area × 100), there was no reduction in lesion area in relation to percent stenosis between 0% and 40% stenosis, indicating that substantial lesions can be accommodated. Above 40% stenosis, there was a dramatic reduction in lumen area, indicating that the limit of compensatory remodeling had been reached. Following Glagov's study, a number of other studies have verified that compensatory remodeling is a generalized phenomenon in both animal models of atherosclerosis and in humans. Although the 40% inflection point varies between studies, indicating that in different models or animals different degrees of atherosclerosis can be accommodated before stenosis develops, failure of remodeling is seen as a major cause of vessel stenosis, irrespective of any changes in atherosclerotic plaque area.

These observations have several important implications. First, they mean that significant atherosclerotic plaques can be accommodated before functionally important stenoses develop. Second, a "near-normal" lumen cross-sectional area detected angiographically does not mean that significant atherosclerosis is not present, and angiography may greatly underestimate the extent of atherosclerosis present. Third, the vessel wall must possess the ability to sense an expanding atherosclerotic plaque, and set in motion a chain of events leading to vessel remodeling. By analogy with other situations where vessels remodel, such as angioplasty restenosis or physiological or pathological changes in blood flow, remodeling is likely to consist of coordinated cell proliferation, migration, and cell death, with matrix synthesis and degradation. This results in the relative movement of tissue from one site in the vessel to another. Finally, as lumen size depends upon both plaque area and IEL area, the onset of clinically apparent stenosis may owe more to the failure of remodeling than a relatively large increase in plaque size. Although the regulation of remodeling is unknown, the ability to promote favorable vessel remodeling after angioplasty or to delay the failure of remodeling in atherosclerosis are now important goals of therapy.

## 1987

One of Mozart's notebooks is sold
for £2.3 million at Sotheby's in London;
Penelope Lively wins the Booker Prize
for "Moon Tiger";
and Hollywood actress
Rita Hayworth dies, aged 68

# Risk of thrombosis in human atherosclerotic plaques: role of extracellular lipid, macrophage, and smooth muscle cell content

*M.J. Davies, P.D. Richardson, N. Woolf, D.R. Katz, J. Mann*

Not all atherosclerotic plaques produce clinically important sequelae. Indeed, most plaques are clinically silent, and the extent of luminal narrowing does not correlate with subsequent unstable angina or myocardial infarction caused by a plaque. The finding that myocardial infarction is due in the vast majority of cases to rupture of the atherosclerotic plaque led to a series of studies examining the composition of plaques, to identify those at highest risk of rupture or those features that predisposed to rupture.

To examine this problem, Davies et al studied intact and ruptured human aortic plaques at postmortem of patients who died of ischemic heart disease within 6 hours of the onset of symptoms. Plaques were assessed for the cross-sectional area of the lipid pool and the area of vascular smooth muscle cells and monocyte/macrophages. Plaques were examined in both the shoulder region and the cap. Plaques that had ruptured showed a larger lipid pool (with >90% of ruptured plaques having lipid pools >40% of the total plaque area), a lower relative and absolute number of smooth muscle cells, and a higher relative and absolute number of monocytes/macrophages. There was also a direct inverse correlation between smooth muscle cell and macrophage numbers, indicating a possible direct cell:cell interaction between the two cell types. Cell changes in the shoulder region and the plaque cap paralleled each other in the same plaque.

This study went an enormous way to identifying the critical components that determine plaque stability, and thus the clinical outcome of atherosclerosis. Thus, until studies of this nature, the conventional view was that smooth muscle cell accumulation was the cause of atherosclerosis, and inhibition of smooth muscle cell proliferation was therefore desirable. In contrast, this study identified that smooth muscle cells promote plaque stability and a benign outcome of atherosclerosis. Thus, smooth muscle cells synthesize collagen and extracellular matrix, improving the tensile strength of the plaque. The smooth muscle cell has since been called the "guardian of the plaque," and disturbances of smooth muscle cell repair may have disastrous consequences for atherosclerotic plaques. Macrophages secrete both collagen and matrix metalloproteinases, directly reducing plaque strength. In contrast, plaque stability can be improved by reducing lipid pools within the plaque, and reducing plaque inflammation. This has shifted treatment away from the aim of regressing atherosclerosis. Indeed, studies of lipid lowering show a <1% reduction in lesion size. In contrast, plaque stabilization is a highly desirable aim, and the likely outcome of the recent studies of β-hydroxy-β-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors. As long ago as 1993, Davies et al predicted that lipid-lowering treatment could reduce lipid accumulation and improve vascular smooth muscle cell content within the plaque. Recent animal studies have confirmed that lipid lowering is associated with an increase in collagen and a decrease in macrophage content of plaques, thus promoting a more stable plaque phenotype.

## 1993

"Unforgiven" wins the Best Picture Oscar;
Roddy Doyle wins the Booker prize for
"Paddy Clarke Ha Ha Ha";
and King Baudouin of Belgium dies, aged 62

# Oxidatively modified low density lipoproteins: a potential role in recruitment and retention of monocyte/macrophages during atherogenesis

*M.T. Quinn, S. Parthasarathy, L.G. Fong, D. Steinberg*

The major initiating event in atherosclerosis is migration of monocytes from the circulating blood into the vessel wall to form resident macrophages, with subsequent accumulation of lipid. Exactly how monocytes are recruited to the vessel wall and retained was, however, unclear. Clearly, adhesion to the endothelial cell surface is important, and endothelial cells had been found to produce chemotactic factors for monocytes. The finding that lipid peroxides and other modified forms of low-density lipoprotein (LDL) are found at high levels in atherosclerotic lesions had focussed attention on these molecules as possible chemotactic agents. However, Steinberg et al had previously shown that oxidatively modified LDL reduced chemotaxis of mouse resident macrophages, questioning the central role of oxidized LDL in atherosclerosis.

In the present study, Quinn et al showed that serum-free medium conditioned by endothelial cells promoted both the migration and movement of freshly isolated human monocytes. This activity could be reproduced by LDL conditioned by endothelial cells, but not by native LDL. $Cu^{2+}$-oxidized LDL was also chemotactic for human monocytes, although other agents that bind to the acetyl LDL receptor on monocytes/macrophages were not. Extraction of the lipid components from LDL revealed the chemotactic activity to reside in the oxidized lipid, but not the lipoprotein component of LDL. As before, the authors demonstrated that oxidized LDL inhibited the chemotaxis of macrophages. Thus, monocytes and macrophages behave in an opposite way to the same stimulus.

This study was an elegant demonstration of the importance of oxidized LDL in both the recruitment and maintenance of vessel wall macrophages in atherosclerosis. These cells are arguably the major cell type that promotes inflammation and lipid accumulation in atherosclerosis, which in turn partly determine clinical outcomes in atherosclerosis *(see Davies et al 1993 on preceding page)*. From the present study, oxidized LDL would be predicted to have at least four roles in atherosclerosis. First, oxidized LDL would induce monocyte recruitment to the vessel wall. Second, accumulation of oxidized LDL in monocytes within the vessel wall by the acetyl LDL receptor would convert these cells to tissue macrophages (foam cells). Third, oxidized LDL would then inhibit foam cell motility and thus egress (thus perpetuating inflammation). Finally, oxidized LDL can induce direct damage to the overlying endothelium, either through actual breaks in the monolayer or by changing transcellular transport processes, thus promoting further LDL emigration. As macrophages can themselves oxidize LDL, a positive feedback loop is established, with sites of lesion formation preferentially accumulating oxidized LDL and macrophage foam cells, both initiating and propagating atherosclerotic lesions. Indeed, lesion-prone regions show increased uptake and degradation of oxidized LDL compared with lesion-resistant regions, even without obvious histological differences. Thus, fatty streaks would be generated by elevated LDL penetration into lesion-prone regions. Oxidation of LDL would then provide the stimulus for the maintenance and propagation of atherosclerosis.

### 1987

Martina Navratilova wins the Women's Singles at Wimbledon for the sixth consecutive time; the ferry "The Herald of Free Enterprise" capsizes near Zeebrugge with the loss of 200 lives; and New Zealand's All Blacks beat France 29-9 in the final of the first World Cup

# Influence of plaque configuration and stress distribution on fissuring of coronary atherosclerotic plaques

*P.D. Richardson, M.J. Davies, G.V. Born*

The demonstration that coronary occlusion is due to plaque rupture (or fissuring) paved the way for a series of studies attempting to identify the characteristics of plaques that rupture, both mechanically (this paper) and histologically *(see Davies et al 1993 on page 165).* This study sought to determine the types of plaques that fissure, the sites of fissuring within the plaque, and the distribution of circumferential stress within the plaque at systole to account for the site of fissuring.

Eighty-five plaques from patients who had died from coronary thrombosis were examined at postmortem. The vast majority (83%) of these plaques contained an eccentric pool of lipid within the intima into which the fissure extended. In nearly two thirds of patients, the plaque had fissured at the junction of the plaque cap with the more normal intima, and one third of plaques had fissured through the plaque cap. Fissure sites were characterized by an accumulation of lipid-laden foam cells. Computer modeling was used to compare differences in shape of plaques and mechanical properties of the artery. Thus, control vessels with uniform mechanical properties around the circumference were compared with a vessel with an easily deformable mass simulating a lipid pool. In addition, circumferential stresses were analyzed in vessels with a deformable mass where the plaque cap region had increasing stiffness relative to the surrounding normal intima, or an artery with a rigid intimal plate, simulating an area of calcification comprising one fourth of the vessel circumference.

The first major point to come from this study is the further iteration that many plaques that rupture are relatively small, and may not extend around the circumference of the vessel. Second, the study demonstrates that fissuring is associated with a lipid pool in one segment of the intima only, with the lateral site of the pool being the most frequent site of rupture. Third, this relationship is likely to be due to the concentration of circumferential stress on the plaque cap, especially at the junction with more normal intima. With increasing plaque cap stiffness relative to intimal stiffness, tensile stress is further increased at this shoulder region. Fourth, although ruptures may occur at sites of maximal stress, inhomogeneities in the vessel wall, such that large local differences in tissue stiffness are adjacent to each other, may promote fracture of the plaque. In this way, matrix degradation from macrophage foam cells may locally weaken the intima, or calcification will result in intimal areas subject to high shear stress. On a smaller scale, concentric layers of fibrosis of atherosclerotic plaques may produce circumferential layers with varying mechanical properties, also concentrating shear stress within the plaque. Both calcification and fibrosis may therefore also promote fissures in the absence of lipid pools within the vessel. On a clinical level, this study further emphasizes the fact that small plaques can be very dangerous, and that plaque fissure is promoted by lipid accumulation, matrix degradation by macrophages, and calcification. The first of these characteristics is now a major target for therapy.

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### 1989

Mikhail Gorbachev
is elected President of the USSR;
the Nobel Peace Prize is awarded
to the Dalai Lama;
and US song writer Irving Berlin dies,
aged 101

# Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man

*S. Yla-Herttuala, W. Palinski, M.E. Rosenfeld, S. Parthasarathy, T.E. Carew, S. Butler, J.L. Witztum, D. Steinberg*

The lipid that accumulates in atherosclerosis is derived from plasma lipoproteins, particularly low-density lipoprotein (LDL). Although monocyte-derived foam cells do not take up native LDL efficiently, modified LDL can be taken up rapidly via the acetyl LDL receptor. Steinberg et al had already found that oxidatively modified LDL could directly promote monocyte emigration into the vessel wall *(see Quinn et al 1987 on page 166)* and inhibit macrophage motility, thus directly promoting macrophage accumulation in lesion sites. However, while this suggests a fundamental role for oxidized LDL in both initiating and promoting atherosclerosis, this evidence resembled a "smoking gun." What was lacking was the demonstration that oxidized LDL is present in the atherosclerotic plaque in vivo.

Yla-Herttuala et al isolated LDL from the aortas of humans and rabbits in the presence of antioxidants, antibiotics, and protease inhibitors, and compared the physical and chemical properties of lesion LDL with in vitro oxidized LDL. Lesion LDL and oxidized LDL both showed higher electrophoretic mobility, higher density, and higher free cholesterol than native LDL. Phospholipid and lipoprotein subfractions were similar in lesion and oxidized LDL, but differed from native LDL. This evidence suggested that lesion LDL is oxidized. This was confirmed by demonstrating that lipoproteins from lesion LDL reacted with antisera that recognize epitopes present in oxidized LDL, but not native LDL. Biologically, lesion LDL and oxidized LDL were similar, producing greater cholesterol esterification and exhibiting greater degradation by macrophages than native LDL. Both lesion and oxidized LDL, but not native LDL, could be recognized by macrophage scavenger receptors, and uptake of lesion LDL could be competed by oxidized LDL. Interestingly, acetyl LDL was less efficient than oxidized LDL in competition experiments, suggesting the presence of another macrophage receptor for oxidized LDL different from the acetyl LDL receptor. Formal proof of the existence of this alternative pathway has since been forthcoming.

Finally, lesion LDL and oxidized LDL (but not native LDL) was chemotactic for monocytes, whereas LDL isolated from normal intima was not.

This study conclusively demonstrates that lesion LDL is chemically, immunologically, and biologically similar to oxidized LDL. Importantly, lesion LDL is taken up rapidly by macrophages, resulting in foam cells, and stimulates monocyte chemotaxis (and presumably inhibits macrophage egress *(see Quinn et al 1987 on page 166)*. Although this study does not allow formal quantification of the extent of oxidation of LDL in vivo, it provides compelling evidence that atherosclerotic lesions contain oxidized LDL. Clearly, the extent of oxidation in any one plaque will be a balance between the penetration of plasma LDL into the vessel wall, the oxidation process, and the uptake and degradation of oxidized LDL by macrophages. The importance of oxidized LDL in atherosclerosis has since been tested in a number of studies of antioxidants in atherogenesis. While these treatments do not regress established lesions, antioxidants can reduce the progression of atherosclerosis in animal models and some human studies.

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## 1989

The "Mini" motor car celebrates its 30th birthday;
Anne Tyler wins the Pulitzer Prize for
"Breathing Lessons";
and Ferdinand Marcos,
former dictator of the Philippines,
dies in exile, aged 72

# Plaque fissures in human coronary thrombosis

*P. Constantinides*

**J Atheroscler Res.** 1966;6:1-17

Historically, coronary thrombosis has been considered in turn to be due to stasis of blood upstream of narrowed vessel segments, a hypercoagulable state in predisposed individuals, or the hemorrhage of plaque vasa vasorum penetrating from the adventitia. Although the possibility that plaque rupture or ulceration may directly promote thrombosis and coronary occlusion had been postulated, until the study by Constantinides there was no direct evidence for this idea, as breaks in the plaque surface had only been demonstrated in a minority of thrombosed vessels. Although this study, published in 1966, may seem trivial in the light of later knowledge, it was truly revolutionary at the time.

Constantinides made a complete serial section study of the occluded coronary artery segment from 20 consecutive cases of coronary thrombosis and compared them with patent segments from 2 cases of coronary wall hemorrhage without thrombosis. Of the 20 coronary thrombosis cases, 16 had recent and 4 had old, organized thrombus. All cases of recent thrombosis showed thrombus anchored to cracks in the atherosclerotic plaque. Over 95% of these thrombi were accompanied by hemorrhages in the surrounding plaque that could be traced to entry of blood through fissures in the plaque. Only minor hemorrhages were noted in association with rupture of vasa vasorum, which were not connected to the lumen. Most of the occlusive thrombus was white thrombus, ie, rich in platelets, fibrin, and leukocytes, with few red thrombi rich in erythrocytes. Despite organization, the old thrombi were similarly attached to plaque fissures. Plaque fissures were usually multiple, of variable size, and in some cases formed large ulcers at the plaque surface. Fissures occurred most frequently at the most proximal section of the occluded segment, but were present throughout the thrombosed arterial segment. Fissures occurred particularly at the margins of the plaque, where plaque and normal vessel wall were joined, and occurred in plaques with thin fibrous caps, with increased necrotic (lipid-rich) regions, or in plaques which showed extensive neovascularization. Fissures were not seen in normal vessel segments proximal or distal to the site of occlusion, or in plaques without thrombosis or hemorrhage.

This study is remarkable for a number of reasons. First, it identifies that coronary thrombosis is caused by plaque ruptures or fissures. Second, it identifies the type of plaques that are prone to rupture (thin fibrous caps, large lipid pools, extensive neovascularization). Third, it identifies the commonest site of rupture (at the shoulder region). Finally, it identifies that plaques are in a dynamic state with fissures and small thromboses being followed by repair and organization. These processes are a common occurrence in plaques that have not caused complete coronary occlusion, and are otherwise clinically silent, and represent the majority of lesions. The truly remarkable feature of this study is that these seminal observations were made over 20 years before the studies of Michael Davies and colleagues that confirmed their validity, and proved that the ulceration/fissure theory of coronary thrombosis favored by Constantinides is correct.

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### 1966

The unmanned Soviet space capsule Luna 9 lands to send the first photographs of the moon's surface; President Charles de Gaulle asks US NATO troops stationed in France to leave the country; and the film Doctor Zhivago, starring Omar Sharif and Julie Christie, is acclaimed at the Cannes Festival

# Atheroma

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## LEAD ARTICLE

The lead article should not exceed **25 standard typed pages** (maximum 8000 words), including an **abstract of no more than 200 words,** no more than **50 references,** and a **minimum of 5 - maximum of 10 illustrations (figures and/ or tables).** A maximum of 5-10 keywords should be included. **The 3 questions for the respondents should be introduced in or after the conclusion.** A separate list of **10 references of "seminal papers"** as well as a separate list of **100 Key References** should be provided.

## RESPONDENT ARTICLES

Respondent articles should not exceed **25 standard typed pages (maximum 2500 words),** including an **abstract of no more than 125 words,** no more than **10 references,** and a **mimimun of 3 - maximum of 5 illustrations (figures and tables).** A maximum of 5-10 keywords should be included.

## SEMINAL PAPER SUMMARIES

Seminal paper summaries take up one page of *Dialogues in Cardiovascular Medicine:* the length of each summary should IMPERATIVELY be comprised between 500 and 600 words, ie, **not exceed 3000 characters.** Summaries that are too short or too long will be returned to the author or edited by the Publisher. No figures, tables or references should be included in seminal paper summaries.