Temporal shifts in clinical presentation and underlying mechanisms of atherosclerotic disease

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Fractures in the foundation of the concept of vulnerable atherosclerotic plaque.

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Abstract

The concept of “the vulnerable plaque” originated from pathological observations in patients who succumbed to fatal acute coronary syndromes. This recognition spawned a generation of research that led to achievement of considerable understanding of how complicated atherosclerotic plaques form and precipitate thrombotic events.

In current practice, an increasing number of patients present with non ST-elevation myocardial infarction (NSTEMI) rather than myocardial infarction (MI) with ST-elevation (STEMI) and survive their first event. The culprit lesions that furnish the pathological substrate for NSTEMI may vary considerably from the so-called “vulnerable plaque.” The shift in clinical presentation of MI and stroke correspond temporally to a progressive change in the characteristics of human plaques away from the supposed characteristics of “vulnerability.” These alterations in the structure and functions of human atherosclerotic lesions may mirror the modifications produced in experimental plaques by lipid lowering, inspired by the “vulnerable plaque” construct.
The shift in the clinical presentations of the acute coronary syndromes mandates a critical reassessment of the underlying mechanisms, proposed risk scores, the results and interpretation of pre-clinical experiments, as well as recognition of the limitations of the use of population data and samples harvested before the application of current preventive interventions.

Key points

- An increasing number of patients presenting with myocardial infarction (MI) are diagnosed with a non ST-elevated myocardial infarction (NSTEMI) rather than a MI with ST-elevation (STEMI) and consequently survive their first event.
- The shift in the clinical presentations of the acute coronary syndromes mandates a critical reassessment of the underlying mechanisms and the concept of the

vulnerable plaque.

- The change in clinical presentation and warrant critical re-assessment of currently applied cardiovascular risk scores, pre-clinical experiments, and the usefulness of population data and samples harvested before the application of current preventive interventions.

Introduction

Temporal changes in the clinical presentation of cardiovascular patients

Since the 1980s the development and increasingly widespread application of effective pharmacologic lipid-lowering and antihypertensive agents and of percutaneous interventions, along with risk factor management have made inroads curbing the worldwide epidemic of cardiovascular disease. Total age-adjusted cardiovascular death rates have fallen by over 50% in most Western countries. This decline may result in part from improved survival after myocardial infarction (MI). Yet, the aging of the population counterbalances some of these advances in combatting coronary heart disease (CHD). As a result, the total economic burden of CHD may actually rise. In the US, between 1970 and 2010 hospital case-fatality for acute MI has dropped from 16.0% to 1.9% and 37.8% to 7.6% for patients aged <65 years and >65 years, respectively (www.nhlbl.nih.gov). The number of hospitalizations for acute MI has also declined significantly: from 53 to 25/10.000 between 1970 and 2010. The decline in death rates due to MI and stroke results in part from a reduction in hospital case fatality. The age- and sex-adjusted incidence of myocardial infarction increased from 274 cases per 100,000 person-years in 1999 to 287 cases per 100,000 person-years in 2000, and it decreased each year thereafter, to 208 cases per 100,000 person-years in 2008, representing a 24% relative decrease. A decline of ST-elevation MI (STEMI) accounts for part of the overall reduction in acute MI. The number of non ST-elevation MI (NSTEMI) and the prevalence of stable angina have changed little. Analyses of data derived from the Top 50 US based cardiovascular hospitals shows that the overall incidence of STEMI decreased by approximately 50% from 29 to 15 per 10.000 Medicare part A beneficiaries [1]. From 2002 to 2011, in the US the proportion of patients with MI due to NSTEMI increased from 52.8% in 2002 to 68.6% in 2011 [2]. The downward trend in STEMI continues not only in most EU based countries but also in the Middle East. For example, in an Israeli study a decline in the incidence of acute coronary syndrome admissions (per-1000 persons) was reported between 2002 vs. 2012 for STEMI: 4.70 vs. 1.38 ($p<0.001$) and non-significant tendency of increase for NSTEMI: 1.86 vs. 2.37 [3].

Here, we will review clinical and pathological determinants that may underlie the observed secular trends in incidence and clinical presentation of myocardial infarction.

Risk factor modification and altered incidence of acute cardiovascular atherosclerotic events.

The explanations for the decline in cardiovascular mortality and disease may differ geographically. The Global Burden of Disease 2010 Study described regional differences in the time trends in the incidence of ischemic heart disease for 21 regions in the world [4]. Lifestyle changes such as improved nutrition and less smoking and/or treatment of risk factors such as blood pressure and high cholesterol have influenced death rates differentially among countries [5]. Experiments of nature and secular economic trends suggest that changes in dietary caloric intake in countries significantly lowers cardiovascular mortality [6].

National regulatory agencies are actively changing policies to reduce the use of unnecessary food additives that influence health. Addition of trans fats to foods and impart a desirable taste and texture. Trans-fat intake associates with higher risk of CHD[7]. National food agencies are restricting the use of *trans* fats in foodstuffs. The Western diet is also high in salt, originating primarily from processed foods. The U.S. Food and Drug Administration has issued proposed guidelines targeting the packaged food and restaurant meals that contain the bulk of American's daily sodium intake. The guidelines aim at bringing daily salt intake to 2,300 milligrams down from a current average of 3,400 milligrams.[8] Estimates suggest that reducing dietary salt by 3 g per day could reduce the annual number of myocardial infarction by more then 50,000. [9]

Governmentally mandated smoking policies have restricted public smoking dramatically, and consequently yielded a major reduction in exposure to passive smoking [10] [11] [12] [13]. The timing of banning of smoking in restaurants resulted in a decrease in hospitals admissions for STEMI between 5-20%. The non-smoking population accounted for this decline, while the number of infarctions in the smoking group remained stable [13].

The widespread increase in the use of cholesterol-lowering drugs, notably the statins, has also altered the risk factor landscape tremendously. The 2013 guideline of the ACC/AHA recommends statin use for all individuals with a risk of >7.5% suffering from a MI or stroke in 10 years [14]. This evidence-based guideline aimed to allocate statins to high-risk patients who

harbor a greater burden of atherosclerosis and followed a process of shared decision making between patient and clinician [15]. The application of this guideline might prevent as many as 450,000 atherosclerotic cardiovascular disease events over 10 years [16].

The effective use of antihypertensive drugs over the last decade has also increased. Blood pressure associates strongly with risk for stroke and for coronary artery disease as well. A strong evidence base supports the efficacy of the management of hypertension in managing stroke risk and that of MI as well. For each 5 mm Hg reduction in systolic blood pressure, cardiovascular risk falls by 17% with little difference according antihypertensive medication class [17 18]. Single and dual antiplatelet therapy another category of pharmaceutical treatment of growing use in secondary prevention and in some high risk individuals in primary prevention in accord with current guidelines may also contribute to reductions in the rate of MI [19 20].

Beneficial changes in diet, life style and medical treatment will have certainly contributed to the decline in STEMI incidence. However, in Western society the average body mass index and diabetes incidence have increased significantly over the last decades that may counterbalance the currently observed decline of MI and stroke. However, it must be noted that obesity increases are slowing [21]. Despite the encouraging drop in age-adjusted cardiovascular mortality and morbidity, the ageing population portends an absolute increase in this burden. In US, some projections predict a rise in total coronary and stroke deaths by 2030 of ≈18%. Deceleration is already observed in the decline of all cardiovascular and stroke mortality rates since 2011.[22] However, the models used for this prediction have engendered debate, since taking into account continued secular trends toward risk factor reduction, coronary mortality may further decrease by 2030 by ≈27% with stroke mortality remaining unchanged. [23]

Controversy persists regarding the body mass index that confers the lowest risk for cardiovascular disease and mortality. Although average body mass index has increased over time in most countries, the overall prevalence of cardiovascular risk factors may also be decreasing among obese individuals over time. Indeed, in 3 Danish cohorts, the body mass index associated with the lowest all-cause mortality increased by 3.3 kg/M^2 from cohorts enrolled from 1976-1978 through 2003-2013.[24]

Age predominates as a risk factor for the development of CHD. Indeed, CHD now first presents in older individuals. Analyses of advanced atheromata show that age associates strongly with elevated inflammatory and proteolytic activity [25].

Diagnosis of MI and reclassification of patients

Only a minority of individuals who present for urgent care for chest complaints actually receive a diagnosis of MI. Early diagnosis is essential to differentiate between patients who require invasive treatment to preserve cardiac function and identify patients who can be discharged with low risk. High sensitivity cardiac troponin assays now serve diagnosis of MI even within the first hour of presentation [26 27 28 29]. The advent of more sensitive troponin measurements will doubtless lead to reclassification of "unstable angina" to NSTEMI, contributing part of the persistence of NSTEMI while STEMI rates decline [30 31]. It merits consideration that European and US guidelines differ in the strength of recommendation regarding the use of high sensitive Troponin in the diagnosis of NSTEMI [32].

Secular trend in clinical presentation and the atherosclerotic plaque.

Improved risk factor control associates with the lower incidence of MI and stroke but does it contribute to the reported atherosclerotic lesion "stabilization" and the subsequent shift in clinical presentation from STEMI to NSTEMI? Serial imaging and pathological studies revealed that statin treatment reduces lipid content and increases the relative collagen content of human plaques, features thought to stabilize lesions [33 34 35–37] These alterations in human plaque morphology correspond well with the changes in lesion characteristics induced by lipid-lowering by diet or by statins in experimental animals [38]. Whether curbs of smoking or the use of antihypertensive drugs produce similar effects on atherosclerotic plaque characteristics has received little attention.

The presenting clinical symptoms of coronary artery disease or cerebral ischemia may reflect the underlying pathological mechanism that leads to the ischemic event. Much of our thinking in this regard over the last decades has focused on the concept of the "vulnerable plaque." Largely based on autopsy studies performed in the latter decades of the 20th century, this formulation has proven highly useful as a guide for research and understanding

of the pathophysiology of the acute coronary syndromes (ACS). Yet, current data suggest that the observed shift in the acute clinical manifestations of coronary heart disease (CHD) reflects a concomitant change in the underlying pathological substrates. These considerations prompt a reevaluation of the relative importance of the so-called "vulnerable plaque" in the current era.

The concept and pathophysiology of the vulnerable plaque

Atherosclerosis causes clinical disease through luminal narrowing or by precipitating thrombi or atheroemboli that impede blood flow to the heart, brain or lower extremities. Histopathological studies of coronary arteries of patients who died of acute MI revealed that large atheroma size, a thin fibrous cap, and the presence of abundant inflammatory cells (mainly macrophages) characterize culprits of fatal thrombotic events [39] [40] [41]. The pathophysiological role of calcium in plaque destabilization may be a double edged sword. Calcified noduli may protrude in the lumen with subsequent thrombus formation [42]. On the other hand calcification may represent "plaque healing" since calcium density relates inversely with CHD and statin use promotes atheroma calcification [43,44]. The pioneering post-mortem studies disclosed that "vulnerable plaques" caused about two-thirds to three-fourths of fatal acute MIs by engendering thrombosis due to a fracture of the plaque's fibrous cap. The "vulnerable plaque" concept has inspired the work of legions of vascular biology researchers. Such lesions became a target for imaging, drug discovery, and quest to mimic plaque rupture in animal experiments.

Yet, despite the value of the "vulnerable plaque" construct in orienting our understanding of the mechanisms of ACS, the concept has encountered challenges, and may apply less to the current era than during the prior period when post mortem studies identified it as a key mechanism of MI. This reevaluation has become timely given the shift in clinical presentations of patients with ACS from a dominance of STEMI to non-STEMI.

Not only have we witnessed a shift in ACS mechanisms, but in stable coronary artery disease as well. We lack sufficient autopsy studies on plaques that cause more stable coronary syndromes, and insights into the pathogenesis of stable coronary syndromes derives mostly from in vivo imaging studies. Compared to patients presenting with acute MI, those with stable angina tend to have less coronary arterial plaque burden, less expansive remodeling,

less abundant evidence of inflammation and neovascularization, and fewer thrombi [45 46].

Furthermore, although analysis at autopsy of culprit lesions responsible for most acute coronary syndromes have revealed plaque rupture, a proportion of occlusive thrombi complicate lesions that lack the typical thin-capped characteristics of ruptured lesions, a morphologic appearance denoted "superficial erosion" [47 48]. These non-ruptured thrombotic lesions occur more frequently observed in women, particularly younger women. Indeed, in females such eroded lesions may cause up to 50% of acute coronary deaths [47]. In males, up to 25% of acute coronary deaths may originate from non-ruptured, eroded lesions. Pathological and in vivo imaging studies suggest that the change in clinical presentation of ACS towards more NSTEMI correlates temporally with a lower prevalence of thrombosis provoked by ruptured plaques. NSTEMI patients have a much lower prevalence of ruptured plaques (43%) than do those with STEMI (71 %) (Table 1). The evolution in the characteristics of atherosclerotic disease may reflect not only secular trends in risk factors and therapeutic interventions, but also demographic shifts in the patients enrolled in studies. Indeed, both trends may contribute to the changes in time in atherosclerosis that we highlight here.

Another major challenge to the "vulnerable plaque" concept arises from the findings that lesions with the histological characteristics associated with "vulnerability" commonly occur in atherosclerotic arteries in the absence of clinically manifest disease. Some ¾ of femoral and coronary atheromata in individuals without acute manifestations show signs of inflammation in their cap and shoulder [49]. Leukocyte activation markers rise in the venous effluent from regions not supplied by the culprit lesions in patients with ACS [50]. Less than 5% of coronary artery plaques characterized as thin-capped fibroatheromata by intravascular imaging cause an acute event during a more than 3 year follow-up period, as shown in the Providing Regional Observations to Study Predictors of Events in the Coronary Tree (PROSPECT) study. [51]. Thus, the vast majority of so-called "vulnerable plaques" are actually quite stable. Moreover, the data reviewed above support the dispersed and multiple nature of such lesions, challenging the validity of approaches that aim to identify and treat locally an isolated vulnerable plaque[52].

The cross-talk between the plaque and the circulation: It takes two to tango

In PROSPECT, circulating C-reactive protein (CRP) concentrations strongly predicted the rate of major adverse coronary events: 13.8% of thin cap fibroatheromatous non-culprit lesions associated with a major adverse event during follow up in for patients with very elevated (>10mg/L), compared with only 1.9% in those with lower CRP (<3 mg/L). The number of high-risk thin cap fibroatheromatous non-culprit plaques did not differ between the high and low CRP groups. This study strengthens the view that in the absence of augmented inflammation revealed by elevation of this marker, few of these lesions provoke an event [53].

Indeed, the thin-capped morphology ascribed to "vulnerable plaques" has limited positive predictive value for the occurrence of an acute event. Fully 105 plaque ruptures in 100 coronary arteries complicated lesions that lacked the so-called "vulnerable morphology" interrogated by virtual histology/IVUS in PROSPECT. During a 3 year follow up the incidence of major adverse cardiac events did not differ between the patients with and those without subclinical, non-culprit plaque ruptures. [54] Thus, the consequences of a plaque disruption depend not only on the 'solid state' of atheroma itself, but involve the presence of other etiological determinants such as the coagulation status of the blood. [55] Coronary CT follow-up studies observed an almost identical number of acute coronary syndromes associated with the presence of high risk plaques (with positive remodeling and low attenuation) and those characterized as "low risk" plaques [56]. In addition, not only the instantaneous morphologic characteristics of a lesion but its dynamics critically influence the propensity to provoke an MI [57 58]. Rapid progression of lesion size could result from intraplaque bleeding, a feature of plaques predictive of future adverse events, but one not included in the current definitions of the vulnerable plaque [59 60].

Time dependent changes in plaque characteristics that underlie cardiovascular ischemic events.

The concept of the vulnerable plaque also requires adjustment as the characteristics of the human plaque evolve in time, in synchrony with the shifts in the clinical presentation and risk profile of patients with ACS. A substantial decline in the incidence of fatal stroke accompanies this evolution in the thrombotic complications of coronary artery plaques [61 62 63]. These tandem temporal changes in coronary and cerebral events suggest a systemic rather than a local change in atherosclerotic disease progression. Athero-Express, an

ongoing Dutch study, has collected atherosclerotic carotid plaques since 2002 [64]. Analyses of retrieved atherosclerotic plaque specimens in the Athero-Express biobank have revealed a clear shift in the characteristics of human atherosclerotic plaques over time [65]. Features that characterize the "vulnerable plaque" such as atheroma size, lipid content, intraplaque hemorrhage, calcifications, and the number of inflammatory cells have decreased markedly in plaques collected more recently. The percentage of carotid plaques with intraplaque hemorrhages decreased from 70% in 2002 to 35% in 2011, concomitantly plaques with large lipid pools fell from 30% to 15% [65] (Figure 2). The time dependent shift from plaques with histological features associated with 'instability' toward those with characteristics generally ascribed to "stable" plaques occurred in lesions retrieved from either symptomatic or asymptomatic patients. Thus, it appears that there is not just a time dependent decline in major cardiovascular event rates, but even within the remaining diseased but event-free or asymptomatic patient group, the character of plaques has shifted in just the last dozen or so years towards features associated with "stability."

Are we facing a paradigm shift in the pathogenetic basis of acute thrombotic atherosclerotic events?

The following observations argue in favor of the proposition that the cardiovascular clinical and investigative communities face a transition that challenges the current view of the prevailing mechanisms that underlie the acute major clinical events due to atherosclerotic disease. 1-The decline in death rate and hospitalisations due to acute coronary syndromes and stroke, 2- The relative increase in NSTEMI versus STEMI in patients presenting with an ACS (Figure 1) and 3-The changes in plaque characteristics of culprit lesions that underlie the acute thrombotic complications that cause stroke and ACS. (Figures 1, 2).

This transition in mechanisms of disease in symptomatic patients coincides with a temporal transition in total atherosclerotic disease burden in the general population. In diagnostic procedures the rate of abnormal SPECT studies impressively declined from 40.9% in 1991 to 8.7% in 2009 [66]. This transition appears to continue, and the search for mechanistic explanations has potentially critical consequences for clinical practice, for public health, and for future research directions.

Plaque erosion: What is in a name?

What morphological, cellular, and molecular features of the non-ruptured atherosclerotic lesion might provoke thrombotic events? As noted above, thrombotic events can arise from fibrous atheromata that lack a thin fibrous cap by a mechanism distinct from fracture generally denoted plaque erosion. The phrase erosion suggests that a dysfunctional or absent layer of endothelium promotes thrombosis. The mechanisms of such an erosive event have received scant attention. Endothelial apoptosis can induce arterial thrombosis in rabbits providing one possible mechanism of plaque erosion [67]. Inflammatory cell recruitment might accelerate this process, although most, but not all, studies find few inflammatory cells within lesions complicated by thrombosis attributed to superficial erosion. [40,68] In vitro experiments found that mast cell release of chymase together with TNF-alpha can induce endothelial apoptosis. [69]. An observational study in human coronary segments showed co-localisation of subendothelial mast cells and intraluminal platelet microthrombi suggesting that desquamation of the endothelium by mast cell proteases induce activation of the endothelium [70]. Endothelial cells can generate endogenous reactive oxygen species (ROS) that subsequently promote cell death. Various pro-inflammatory and pro-atherosclerotic factors such as Angiotensin II and TNF-alpha can induce ROS generation by endothelial cells [71].

The neutrophil cell lineage may also participate in plaque thrombosis without rupture, although likely in a second wave, after initiation of the process [72]. Neutrophils contain abundant myeloperoxidase, a pro-oxidant enzyme that circulates at higher concentrations in patients with acute coronary syndrome due to an eroded culprit lesion compared with patients with a ruptured culprit plaque as defined by optical coherence tomography. In addition, luminal thrombi on eroded coronary artery plaques at postmortem examination contain more myeloperoxidase-positive cells than thrombi caused by ruptured plaques, suggesting the presence of neutrophils [73]. Neutrophils can elaborate strands of DNA, called neutrophil extracellular traps (NETs.) These extracellular DNA strands can promote primary and secondary hemostasis by inducing platelet aggregation and activation of coagulation. In addition, NETs may inhibit fibrinolysis and thereby enhance clot stability. Neutrophil recruitment to the vessel wall, and subsequent NET generation can contribute to a highly pro-coagulant environment that could amplify, propagate, and sustain arterial thrombi [74].

Less lethal coronary artery disease and altered mechanisms of thrombotic events: possible clinical and research implications

The evolving characteristics of atherosclerotic plaques that cause clinical manifestations in contemporary practice provides an alert that the research community needs to update thinking regarding the current clinically relevant mechanisms of the thrombotic complications of atherosclerosis. A multitude of experimental studies, most often using genetically modified mice with exaggerated cholesterol concentrations, refer to the effects of various genetic or pharmacologic manipulations on what publications often refer to as "stable" or "unstable" plaque "phenotype." The current human pathological and clinical findings reviewed here cast serious doubt on the applicability of such studies to contemporary clinical practice. Indeed, the ongoing shift in the incidence in atherosclerotic disease manifestations, and the responsible pathological substrate require consideration in the interpretation of many past basic, translational and clinical studies. Extrapolation of observations from the last century, obtained in a bygone era of therapy and risk factor exposure, warrants a re-evaluation of the key current questions for research, and the interpretation of experimental results. The section below discusses the potential implications of the currently shifts in clinical manifestations and pathogenesis of atherosclerotic disease.

Population and cohort based biobanks initiated over 10 years ago.

The decline in age-adjusted incidence of events due to atherosclerosis, the altered risk profiles, and the increased use of highly effective preventive medications calls into question the relevance to today's disease of plaque or blood specimens banked frozen for future research purposes in past decades. The translational value of observations made on such materials for current practice raise even further concern if both the sampling of specimens and the monitoring of cardiovascular events took place more then a decade ago. Circulating biomarkers that add to the prediction of the progression of atherosclerotic disease and inform regarding the effects of novel therapies still comprise an unmet need [75 76 77] The concept that acute atherosclerotic events commonly complicate highly inflammatory and proteolytic plaques has driven many biomarker discovery programs. Many proposed

biomarkers with predictive value for MI such as myeloperoxidase [78], high sensitive C reactive protein, and soluble ICAM [79] arise from the idea that vascular inflammation participates pivotally in the pathogenesis of cardiovascular events. While this concept has served the community well, and derives support form a large body of consistent studies, this formulation may apply less to a growing group of patients with ischemic heart disease receiving *current* standard of care treatments. In Utrecht, a study exploring the determinants of cardiovascular events has enrolled patients with any kind of manifestations of atherosclerotic disease since 1995 [80]. This study observed a consistent decline in CRP concentrations over time (Figure 3). These considerations indicate that the time window of enrollment and of blood sampling can influence profoundly the observational associations between biomarkers, the burden of atherosclerotic disease, and clinical events.

Imaging of the atherosclerotic plaque at risk

The pathological observations that delineated the characteristics of the rupture prone plaque, the thin capped fibroatheroma, has inspired a legion of quests to develop imaging modalities to assess such lesions in vivo. Ultrasound based "virtual histology" [81], Raman spectroscopy [82], thermography [83] near-infrared spectroscopy [84] or optical coherence tomography [85] [86], and a variety of molecular imaging strategies have all undergone development seeking to detect the inflammatory thin cap fibroatheroma, the type of plaque supposed to entail a high risk for a thrombotic event based on the pathological observations from the last century. Non-invasive magnetic resonance imaging can identify intraplaque hemorrhage, a plaque characteristic that may predict future adverse cardiovascular events and progression of luminal narrowing [87] [60]. Imaging of features that associate with the classically defined thin cap fibroatheroma may help to assess the efficacy of plaque stabilizing drugs. Yet, the community should take into account secular trends in atheroma as therapy has advanced. For example, atorvastatin vs. pravastatin produced a reduction in carotid intima-media thickness in the ASAP study reported in 2001, but the addition of ezetimibe did not decrease this variable in the ENHANCE trial reported in 2008. At present carotid intima media thickness may be challenged when used as a measure of drug efficacy, but this result might have arisen because of application of standard of care in the interim

lowered the baseline intima-media thickness to the "normal" range, obscuring the possible effect of the additional therapy. [88] [89] The lower incidence of STEMI and the concomitant increase in less lipid laden and inflamed plaques in the statin era raise similar notes of caution regarding the interpretation of and goals of imaging studies. Others share this view, refer to "the myth of the vulnerable plaque", and propose that the management of patients at risk of MI mandates a greater focus on atherosclerotic disease burden than on features of individual plaques [52], an inference that derives support from studies with coronary computed tomography angiography in patients with non-obstructive coronary artery disease [90].

Cardiovascular risk scores used to predict the absolute risk of cardiovascular events

Various proposed cardiovascular risk scores predict the risk for future primary events and stratify patients into low, intermediate and high-risk categories. As a community we have generally targeted the most aggressive risk factor management to higher risk individuals. Risk scores such as the Framingham risk score, have served as a comparator to explore the added value of novel biomarkers on top of traditional risk factors for the prediction of coronary heart disease [91]. The arguments presented here, notably regarding the trend toward less STEMI and altered characteristics of culprit plaques, may impact the current clinical relevance of the most widely applied cardiovascular risk scores. Most population based cohorts that have provided event rates for risk assessment in these studies underwent a follow up of at least 10-12 years, and included individuals whose had blood sampled before 1990 or as far back as the 1960s. At inclusion these individuals were almost certainly exposed to different risk factors, medical treatments, more passive smoking and nutritional and other life style measures than contemporary cohorts. Moreover, clinical endpoints in prior eras included more STEMI, higher case fatality rates for ACS, and as argued here, an underlying pathological substrate that has shifted as compared to nowadays. The consequence of the application of cohorts sampled in prior eras for risk score assessment has contributed to the recent debate regarding the use of statins based on the new AHA-ACC-ASCVD risk scores [92]. The newly developed risk scores proposed by the ACC/AHA guidelines may overestimate cardiovascular events by 37% to 154% in men and 8% to 67% in women when discrimination and calibration was compared with other risk scores and the

actual observed events [93]. Cardiovascular risk scores should undergo external validation in current populations before their clinical implementation. One contribution to this controversy could be the use of external validation cohorts sampled more recently than those used to derive the risk prediction algorithm, and that therefore reflect secular improvements in overall health and lifestyle patterns [92]. Indeed, the cohorts used for the newly developed ACC-AHA risk prediction models originated from cohorts assembled before 1990. Risk prediction algorithms should not only discriminate between individuals with and without disease, but must also calibrate well so that predicted risk estimates match as closely as possible the observed risk in external populations [94]. In a recent editorial on a report that clearly showed that the ACC/AHA Pooled Risk Equation substantially overestimates the 5 year risk of atherosclerotic cardiovascular events[95]. Indeed, Blaha has argued that risk score calibration is among the most important issues in preventive cardiology[96]. The validation of risk instruments should use cohorts that reflect current practice as closely as possible. This goal presents a challenge since accumulation of events requires a follow up period, such that external validation uses cohorts that enrolled more than one decade ago.

Implications for the interpretation of animal experiments

The slow progression of human atherosclerosis hampers the study of the natural history of this disease, which would ideally involve extensive serial examinations. To provide insight into mechanisms of pathogenesis and the effects of therapies, laboratory studies generally use *in vitro* cell-based approaches or experiments in animals. The generation of genetically modified mice on an atherosclerotic background exponentially increased the published papers on genes that may influence plaque growth and lesion characteristics [97 98]. The interpretations of many interventions in mice often refer back to the early pathological descriptions of the "vulnerable plaque" deemed responsible for triggering most thrombotic occlusive events in humans [99]. Despite its undeniable utility for probing specific molecular mechanisms, atherosclerosis-prone mice differ in a multitude of important respects from humans with arterial disease [100 101]. A rigorous assessment of the clinical translatability of the results of investigations in atherosclerotic mice requires careful and deliberate consideration of these concerns. In particular, the profound effects of current clinical standard of care on human atherosclerotic lesions and their clinical consequences may

amplify the distance between the exaggerated experimental conditions usual in mouse studies of atherosclerosis and patients with this chronic condition now usually receiving multiple medical interventions and often harboring co-morbidities not modeled in mice. Few established experimental approaches described in animals aim to mimic aspects of spontaneous luminal thrombus formation on stable atherosclerotic plaques. [102]

Conclusions

In medicine, as in many other pursuits, we should strive to keep eyes on a moving target. The edifice of the "vulnerable plaque" arose from a foundation as firm as any in science. We celebrate justly the strides we have made in mastering the lipid-rich atheromatous plaque, from both a pathophysiologic and therapeutic perspective. The benefits that have emerged from these advances for public health merit considerable celebration. The last decades we have enjoyed the rare privilege of witnessing extraordinary inroads against atherosclerotic disease, akin to the transformative effect of the introduction of antibiotics on infective diseases.

Yet, the very measures that have enabled our extraordinary success in improving clinical outcomes in those at risk, have altered the disease itself. Moreover, the improved understanding and treatment of classical risk factors for atherosclerosis, has occurred in the face of a countervailing worldwide increase in obesity and dysmetabolism that may drive the next wave of epidemic cardiovascular disease. These concerns preclude premature celebration of victory against atherosclerotic disease. To confront the residual burden of disease in an era of highly effective LDL lowering, societal measures to combat tobacco abuse, and the widespread availability of strategies for management of hypertension, we must prepare to wage battle against the disease of today and tomorrow, not that of yesteryear. We can take heart in our success in lowering total plaque burden and taming the "vulnerable plaque," and now turn our attention to combatting residual risk in the current era with the same energy and enthusiasm that brought us to this "tipping point" in the disease. To meet this challenging objective we will need to reconsider the implications for current practice of findings collected before new millennium, and all of the changes in therapy and the risk factor environment that have ensued. Biomarker and drug target discovery programs should interpret with caution data derived from samples that were

obtained >10-15 years ago, risk scores should be calibrated studying cohorts that resemble the current population, and development of imaging modalities should reach beyond the characteristics of the so-called vulnerable lesion, and embrace more fully gauging total plaque burden.

	Clinical presentation	% with rupture	% no rupture	Number included	ref
Pathology	Death due to ACS	75	25	291	[48]
Pathology	Sudden death	56	44	50	[47]
Pathology	Sudden death	69	31	59	[103]
OCT	STEMI	72	28	53	[104]
OCT	STEMI	70	30	40	[105]
OCT	STEMI	64	36	47	[106]
OCT	STEMI	83	17	24	[107]
OCT	STEMI	66	34	95	[108]
OCT	STEMI	72	28	53	[109]
OCT	STEMI	71	29	102	[110]
OCT	STEMI	64	36	14	[111]
OCT	STEMI	60	40	62	[112]
IVUS/atherectomy	STEMI	83	17	54	[113]
OCT	NSTEMI	49	51	110	[104]
OCT	NSTEMI	19	81	43	[114]
OCT	NSTEMI	47	53	49	[105]
OCT	NSTEMI	57	43	92	[106]
OCT	NSTEMI	46	54	83	[107]
OCT	NSTEMI	40	60	40	[109]
OCT	NSTEMI/UA	33	66	24	[111]
IVUS/atherectomy	NSTEMI	25	75	40	[113]

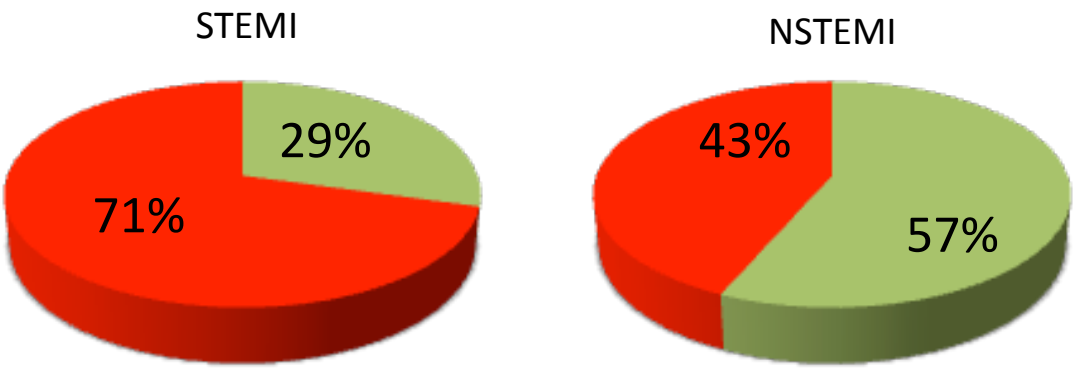


Table 1 and summarizing figure. There is an evident shift in clinical presentation over time with lower incidence of STEMI and an increased proportion of patients presenting with NSTEMI. This table reflect subsequent changes in plaque characteristics underlying luminal occlusive thrombosis. Pathology and optical coherence tomography (OCT) studies report a plaque rupture in 71% of the lesions of patients presenting with sudden death, death due to an acute coronary syndrome or presenting with STEMI (figure depicted red). In NSTEMI patients approximately 43% of the culprit lesions reveal a plaque rupture on OCT. Data in the figure are obtained and calculated from references mentioned in the Table.

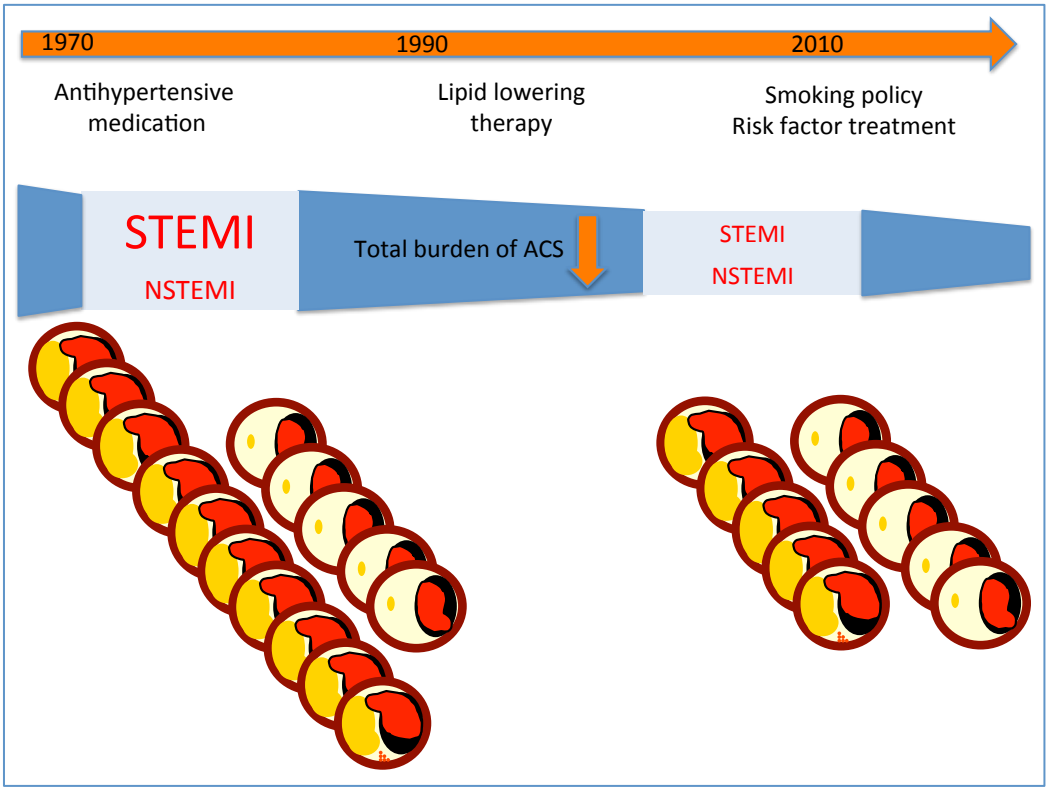
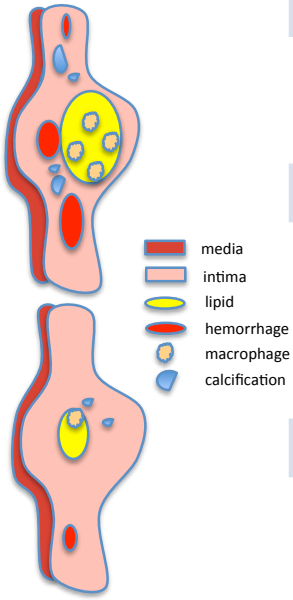


Figure 1. A decline in total death rate and hospitalizations following ACS and acute stroke has occurred over time. A fall in the incidence of STEMI accompany these clinical trends and a contemporaneous shift in the characteristics of human atherosclerotic plaques away from those associated with "vulnerability" or plaque rupture. The rise in use of antihypertensive medication, cholesterol lowering treatment, anti platelet therapy and risk factor management has likely contributed to this secular trend. Banning of smoking by governmental policy strongly influenced the risk of passive smoking.



Shifts in the composition of human atheromata from 2002-2003 to 2010-2011

Lipid	Macrophages	Hemorrhage	Calcification
(% with >40 % fat)	(% of plaque cross sectional area)	(% with intraplaque)	(% with heavy or moderate staining)
33%	0.41%	74%	52%
2002-2003			
14%	0.09%	38%	24%
2010-2011			

Figure 2. The changes in plaque composition over time observed in carotid arterial plaques that have been dissected by endarterectomy.

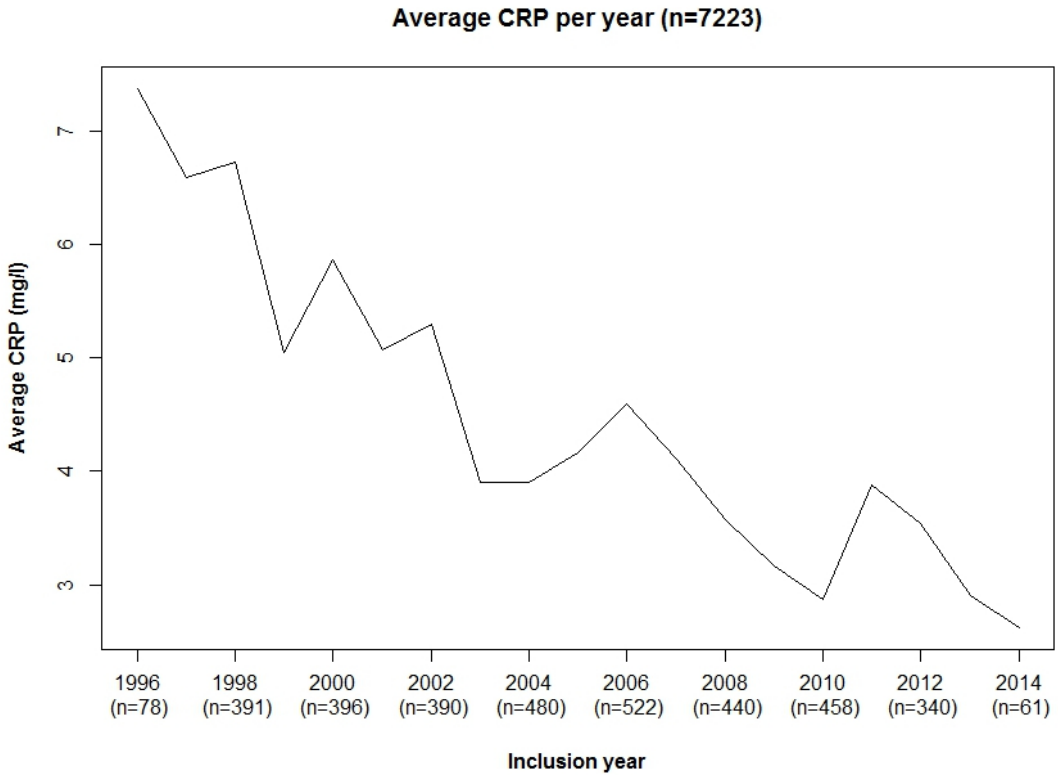


Figure 3. C-reactive protein levels in patients who have been included in the SMART study and have been diagnosed with either carotid, coronary or peripheral artery disease in the University Medical Center Utrecht [58].

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