Newer Risk Markers and Surrogate Endpoints in Atherosclerosis Management

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Summary: Risk markers that allow improved and individualized assessment of atherosclerotic disease risk and response to treatment are needed. Current candidate markers include cell adhesion molecules, such as intercellular adhesion molecule-1 and E-selectin, and inflammatory markers, such as C-reactive protein; advances in genomics and proteomics will suggest additional candidate markers. Noninvasive imaging procedures such as electron-beam computed tomography and magnetic resonance imaging also show considerable promise for monitoring disease status and response to treatment, and ultimately could provide surrogate endpoints for clinical trials of therapeutic interventions.

Key words: atherosclerosis, intercellular adhesion molecule-1, E-selectin, C-reactive protein, electron-beam computed tomography, magnetic resonance imaging

Introduction

Availability of markers of atherosclerotic plaque status and response of atherosclerosis to treatment would be tremendously useful in cardiology practice. Currently, symptoms and stress testing can detect limitation of blood flow leading to myocardial ischemia, whereas direct assessment of atherosclerotic disease status depends on expensive and invasive

measures, such as quantitative angiography and intravascular ultrasound. Although low-density lipoprotein (LDL) cholesterol targets may be useful for large populations, the LDL cholesterol level does not tell us whether we have “halted” atherosclerosis in an individual. Multiple epidemiological studies have shown that susceptibility to coronary heart disease at any given LDL cholesterol level is highly variable and dependent on genetic and environmental factors. Ongoing research suggests that there is considerable potential for development of a number of blood tests and noninvasive measures that may permit accurate assessment of disease burden and response to treatment, some of which may prove useful as surrogate endpoints in clinical trials.

Cell Adhesion Molecules and Inflammatory Markers

Part of the optimism regarding development of new risk markers has been generated by the considerable recent advances in our understanding of the pathophysiology of atherosclerosis. Development of disease involves the interaction of leukocytes with the vascular endothelium: capture of leukocytes by the endothelium, leukocyte adhesion to the endothelium, and leukocyte transmigration into vascular tissue are mediated by a number of cell adhesion molecules (CAMs), including integrins and selectins, as well as other chemoattractant substances within the vascular tissue.¹ High concentrations of CAMs have been consistently found in areas of plaque development, prompting analysis of their relation to clinical outcomes associated with coronary heart disease (CHD).²

This was first examined in the Atherosclerosis Risk in Communities (ARIC) study, a prospective trial of 15,792 African-Americans and Caucasians, aged 45 to 64 years. In this study, levels of circulating adhesion molecules were assessed in 204 patients with incident CHD events (fatal or non-fatal myocardial infarction [MI] or surgical revascularization), 272 with carotid artery atherosclerosis on B-mode ultrasound, and 316 age- and gender-matched control subjects without carotid artery atherosclerosis. Subjects in the highest quartile for intercellular adhesion molecule-1 (ICAM-1) levels had an odds ratio for CHD events of 5.53 (95% confidence interval [CI] 2.51–12.21) compared with subjects in the lowest quartile

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after adjustment for race, age, gender, body mass index, hypertension, diabetes, total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, cigarette smoking, von Willebrand factor, fibrinogen, and white blood cell count.³ Subjects in the highest quartile for ICAM-1 levels also had an odds ratio for carotid artery atherosclerosis of 2.64 (95% CI 1.40–5.01) compared with the lowest quartile, and those in the highest quartile for endothelial-leukocyte adhesion molecule-1 (E-selectin) had an odds ratio of 2.03 (95% CI 1.14–3.62) for carotid artery atherosclerosis compared with those in the lowest quartile. No significant association was found between vascular cell adhesion molecule-1 (VCAM-1) level and either CHD events or carotid artery atherosclerosis.

Assessment of risk for first MI in the Physicians' Health Study provided further evidence of an association between ICAM-1 and CHD.⁴ The investigators demonstrated that risk was increased in patients with higher baseline soluble ICAM-1 levels, and a subanalysis of subjects with elevated ICAM-1 levels (> 260 ng/dl) showed that this increase in risk became significant after 2 years, with relative risk for MI doubling after 4 years.

More limited data are available on whether CAMs may be useful in assessing response to therapy. In a study of 27 patients with hypertriglyceridemia, treatment with n-3 fatty acid, which reduced triglyceride levels by 47%, was associated with significant decreases in both soluble ICAM-1 levels (9%) and soluble E-selectin levels (16%)⁵; decreases in these CAMs were greatest in patients with diabetes. A small study in patients with very high LDL cholesterol levels found that aggressive lowering of LDL cholesterol (by 51%) was associated with a significant reduction in soluble E-selectin levels, but no such improvements were seen with respect to ICAM-1 or VCAM-1.⁶ The findings of other studies have been more ambiguous with respect to the relationships between adhesion markers and CHD.⁷ For example, Sampietro *et al.* demonstrated an improvement in carotid artery geometry on ultrasound and a reduction in soluble E-selectin levels after 12 weeks of atorvastatin treatment in 20 hypercholesterolemic patients with or without cardiovascular disease; however, no significant changes in ICAM-1 or VCAM-1 levels were observed.⁷

A large number of studies have recently focused on an older marker of inflammation, C-reactive protein, which now can be measured with newer high-sensitivity assays (hs-CRP). One of these studies, a subgroup analysis of the Cholesterol and Recurrent Events (CARE) trial, compared 391 patients who had a recurrent coronary event following initial MI with 391 age- and gender-matched controls who did not.⁸ They found that the risk of recurrent events was markedly increased in placebo patients whose CRP and serum amyloid A (SAA) levels were $\geq$ 90th percentile compared with placebo patients with lower CRP and SAA levels and pravastatin patients with levels $\geq$ 90th percentile (Fig. 1).⁸ In a separate study, assessment of CRP levels at baseline and at 5 years in 472 patients from the CARE trial who remained free of recurrent events showed that pravastatin recipients had significantly lower mean ($p = 0.002$) and median ($p = 0.007$) CRP levels after

5 years. In pravastatin recipients, the mean level decreased from 0.38 mg/dl at baseline to 0.31 mg/dl and the median level decreased from 0.23 to 0.19 mg/dl, whereas in the placebo group the mean level increased from 0.36 to 0.43 mg/dl and the median level increased from 0.24 to 0.25 mg/dl.⁹

Data from studies in the Physicians' Health Study population indicate both that hs-CRP is predictive of future MI in apparently healthy middle-aged men and that the combined use of hs-CRP and lipid measurements has an additive effect in predicting future MI.¹⁰ Relative risk is markedly increased in patients with both elevated hs-CRP values and elevated total cholesterol: HDL cholesterol ratio compared with the relative risks associated with either factor alone (Fig. 2).

Although hs-CRP testing appears promising and has been approved for use, there are also data that indicate that its predictive effect is reduced when other potential risk factors are taken into account. In particular, analysis of risk ratios associated with CRP levels in the West of Scotland Coronary Prevention Study (WOSCOPS) population showed weakening of predictive value after adjustment for lipoprotein-associated phospholipase A₂, white cell count, and fibrinogen level and further weakening after adjustment for age, systolic blood pressure, triglyceride levels, and LDL and HDL cholesterol levels (Fig. 3A).¹¹ This study instead identified lipoprotein-associated phospholipase A₂ as a significant predictor of coronary events, since risk ratios associated with increased levels of this marker were not diminished by adjustment for either (1) CRP, white blood cell count, and fibrinogen, or (2) age, systolic blood pressure, triglycerides, LDL cholesterol, and HDL cholesterol (Fig. 3B).

It is likely that the coming era of risk marker investigation will involve assessment of a wide variety of potential markers and will include development of panels of markers for simul-

FIG. 1 Relative risk of recurrent coronary events in patients post myocardial infarction from the Cholesterol and Recurrent Events (CARE) study (391 with and 391 without recurrent events) according to (1) assignment to placebo or pravastatin and (2) whether both C-reactive protein (CRP) and serum amyloid A (SAA) levels were below the 90th percentile or at/above the 90th percentile for the population studied. Reproduced with permission from Ref. No. 8.

FIG. 2 Relative risk of myocardial infarction in apparently healthy men in the Physicians' Health Study according to baseline levels of risk markers. t-PA = tissue-type plasminogen activator, TC = total cholesterol, HDL = high-density lipoprotein, hs-CRP = high-sensitivity C-reactive protein. Adapted from Ref. No. 10.

taneous evaluation. Candidates for evaluation will continue to include molecules identified on the basis of their biologic importance in the atherosclerotic disease process, but are also likely to include genetic markers. Advances in genomics have provided technology that permits analysis of gene expression and gene profiling in different cell types, including those found in the atherosclerotic lesion, as well as assessment for genetic polymorphisms associated with CHD. Similarly, advances in proteomics, combined with mass spectroscopy microsequencing technology, may enable investigators to rapidly profile and indentify the differentially expressed proteins in cells and lesions.

Noninvasive Imaging

Both electron-beam computed tomography (EBCT) and magnetic resonance imaging (MRI) hold promise for use in assessing atherosclerosis disease status and response to treatment; atherosclerotic progression detected by these methods

FIG. 3 Unadjusted and adjusted risk ratios for coronary events according to C-reactive protein (CRP) level (A) and lipoprotein-associated phospholipase A₂ level (B) in patients in the West of Scotland Coronary Prevention Study (WOSCOPS) population. RR = relative risk, LPA PLA₂ = lipoprotein-associated phospholipase A₂, LDL-C = low-density lipoprotein cholesterol, HDL-C = high-density lipoprotein cholesterol. Reproduced with permission from Ref. No. 11.

FIG. 4 Initial and final calcium-volume scores on electron-beam computed tomography in patients not receiving statin treatment (Rx) with average LDL cholesterol (LDL-C) of 147 mg/dl and in patients receiving statin treatment with LDL-C levels ≥ 120 mg/dl or < 120 mg/dl. NS = not significant. ■ = Initial, ▨ = final. Reproduced with permission from Ref. No. 12.

may ultimately prove to be a reliable surrogate endpoint for use in clinical trials. Electron-beam computed tomography can be used to visualize and quantify coronary artery calcification, a parameter that corresponds closely with the total burden of atherosclerotic plaque. A study in 149 patients without a history of CHD showed that over 12 to 15 months of follow-up, mean calcium volume score as assessed by EBCT increased by 52% in a group of patients with average LDL cholesterol levels of 147 mg/dl who did not receive statin treatment, and by 25% in patients who had LDL cholesterol levels ≥ 120 mg/dl who did receive statin treatment. However, in patients with LDL cholesterol levels < 120 mg/dl receiving statin treatment, the score decreased by 7% (Fig. 4).¹² Magnetic resonance imaging can be used not only to assess vessel lumen diameter, but also to quantify plaque volume and lipid, cellular, and fibrous components of plaque. With improvement in the specific technology, this technique may provide a method for assessing plaque composition and monitoring response to treatment.^{13,14}

Conclusion

Alternative atherosclerotic risk markers that can be evaluated by blood testing or other noninvasive means are needed to improve risk assessment, monitoring of disease status, and response to treatment. Candidates include the inflammatory markers discussed here, as well as noninvasive imaging techniques; continuing advances in molecular biology, genomics, and proteomics will identify additional candidate markers. It is likely that in the not too distant future, we will be able to assess risk and monitor disease status and response to treatment on an individualized basis using panels of multiple risk markers.

Improved methods for monitoring disease status and response to treatment will also ultimately provide us with new surrogate endpoints for trials of therapeutic intervention. Al-

though progression of atherosclerosis on coronary angiography is a reliable surrogate marker of risk of clinical events, methods that are less expensive and invasive than angiography are clearly needed. Clinical endpoint trials have provided important information about the effectiveness of lipid-modifying therapies in preventing CHD events. However, there are several important limitations that could be avoided by identifying and using appropriate surrogate endpoints.¹⁵ For example, clinical endpoint trials require large sample sizes and lengthy follow-up periods and are expensive to perform; in addition, there are ethical issues regarding withholding treatment from patients receiving placebo for the duration of the trial. The findings of such trials, moreover, are relatively insensitive in determining therapeutic effects on plaque status or disease progression: although clinical events are usually caused by plaque rupture/erosion with accompanying thrombosis, the processes of plaque rupture and erosion are most often asymptomatic. Finally, clinical endpoint trials do not provide the information needed for individualization of therapy because they do not directly measure treatment effects on the underlying disease processes of atherosclerosis and thrombosis.

Currently, there is considerable hope that methods such as EBCT and MRI will permit adoption of new surrogate endpoints in clinical studies. However, it must be remembered that such endpoints must be biologically plausible—that is, related to the processes of atherosclerosis, plaque instability, and thrombosis—and must be altered by therapies that decrease the endpoint for which that marker is a substitute. Definitive demonstration of the ability of noninvasive imaging techniques and/or new surrogate markers to satisfy these criteria is awaited.

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