New Risk Factors for Atherosclerosis and Patient Risk Assessment

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Abstract—Advances in our understanding of the ways in which the traditional cardiovascular risk factors, including standard lipid (eg, total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol) and nonlipid (eg, hypertension) risk factors, interact to initiate atherosclerosis and promote the development of cardiovascular disease have enhanced our ability to assess risk in the individual patient. In addition, the ongoing identification and understanding of so-called novel risk factors may further improve our ability to predict future risk when these are included along with the classic risk factors in assessing the global risk profile. This review briefly summarizes the evidence that some newer risk factors, including impaired fasting glucose, triglycerides and triglyceride-rich lipoprotein remnants, lipoprotein(a), homocysteine, and high-sensitivity C-reactive protein, contribute to an increased risk of coronary and cardiovascular diseases. (Circulation. 2004;109[suppl III]:III-15–III-19.)

Key Words: atherosclerosis ■ novel risk factors ■ risk assessment ■ triglycerides

In the last decade, substantial improvements have occurred in the assessment of cardiovascular risk. A better appreciation of the atherogenic effects of well-known cardiovascular risk factors has been accompanied by an understanding that the sum of these factors—ie, the global risk profile—provides better predictive power than any single risk factor. In addition, a number of more recently identified and less well-known factors have received intense investigation over the past few years. In the present review, we briefly summarize evidence of the prognostic value of several of these newer risk factors. These include both lipid and nonlipid variables, such as metabolic factors (eg, impaired fasting glucose), thrombogenic/hemostatic factors (eg, fibrinogen), and inflammatory markers. [Note: Other potential new lipid-related markers of risk, such as small, dense low-density lipoprotein particles, oxidized low-density lipoprotein, and apolipoprotein B, are discussed elsewhere in this supplement in the article, “Atherogenic Lipoprotein Particles in Atherosclerosis.”]

So far, there is no universal agreement on the exact placement of the various cardiovascular risk factors and risk markers within the old–new continuum; a suggested classification, including an intermediate term ("old/new"), is outlined in the Table. In addition, there is some controversy about whether or not to include some of these factors in the various scoring schemes currently used to calculate a person’s long-term risk, which, in turn, may help determine appropriate treatment for persons without clinically established disease. For example, the version of the Framingham risk point scores adopted by the U.S. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III [ATP III]) is based on the traditional (old) risk factors of age, sex, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, systolic blood pressure, and smoking.1 By contrast, in the more recent Prospective Cardiovascular Münster (PROCAM) simple scoring scheme, 8 risk variables are identified: age, LDL cholesterol, smoking, HDL cholesterol, systolic blood pressure, family history of premature myocardial infarction (MI), diabetes mellitus, and triglycerides.2 Despite the lack of agreement, however, continued focus on newer factors is warranted, as they may further improve our ability to predict future risk and determine treatment when they are included along with the classic risk factors in the global risk profile.

Metabolic Risk Factors

Diabetes Mellitus, Impaired Glucose Tolerance, Impaired Fasting Glucose

Epidemiological studies have shown that patients with diabetes mellitus and glucose intolerance are at increased risk for coronary heart disease (CHD). In the first 20 years of the Framingham Heart Study, the incidence of cardiovascular disease among men with diabetes was twice that among men without diabetes. Among women with diabetes, the incidence of cardiovascular disease was 3 times that among women...
Old, Old/New, and New Risk Factors for Atherosclerosis

<table>
<thead>
<tr>
<th>Old</th>
<th>Old/New</th>
<th>New</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (men &gt; women)</td>
<td>High-normal blood pressure</td>
<td>Apolipoprotein B; apolipoprotein A-I</td>
</tr>
<tr>
<td>Age</td>
<td>Metabolic syndrome</td>
<td>Triglycerides; triglyceride-rich lipoprotein remnants</td>
</tr>
<tr>
<td>Family history of premature cardiovascular disease</td>
<td>Diabetes mellitus; impaired glucose tolerance; impaired fasting glucose</td>
<td>Small, dense LDL; oxidized LDL; antibodies against oxidized LDL</td>
</tr>
<tr>
<td>Total cholesterol; LDL cholesterol; HDL cholesterol (negative risk factor)</td>
<td></td>
<td>Lipoprotein(a)</td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td>Homocysteine</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td>High-sensitivity C-reactive protein</td>
</tr>
<tr>
<td>Overweight/obesity</td>
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HDL indicates high-density lipoprotein; LDL, low-density lipoprotein.

without diabetes. Data from Framingham confirmed that long-term hyperglycemia increases the risk of microalbuminuria. The simultaneous development of microalbuminuria, type 2 diabetes, and coronary atherosclerosis in the course of follow-up is consistent with the hypothesis that these phenomena share a common pathophysiological pathway. In particular, microalbuminuria and silent myocardial ischemia at baseline had a clearly predictive value for future CHD in (asymptomatic) patients with type 2 diabetes.

The Framingham Offspring Study has evaluated the impact of impaired glucose tolerance (IGT), impaired fasting glucose (IFG), and type 2 diabetes mellitus versus normal glucose tolerance (NGT) on the process of atherosclerosis. Of 325 subjects aged 31 to 73 years (one half of whom were men), 11.2% had IFG/IGT, 9.9% had diabetes (2.8% with previously diagnosed diabetes), and 14.5% had insulin resistance. Compared with NGT, subjects with IFG/IGT were more likely—and those with diabetes were significantly more likely—to have subclinical coronary atherosclerosis, as defined by coronary artery calcium scores obtained by electron beam computed tomography. Subjects with previously diagnosed diabetes had a 3 times greater risk than did subjects newly diagnosed based on impaired oral glucose tolerance (odds ratios [OR]: 6.0 versus 2.1). Subjects with insulin resistance were twice as likely to have subclinical coronary atherosclerosis as those without insulin resistance. However, this association was weakened after adjusting for other risk factors (cigarette smoking, total/HDL cholesterol ratio, and systolic blood pressure). This study reconfirms that persons with diabetes have an elevated burden of subclinical coronary atherosclerosis.

The NCEP considers diabetes a CHD risk equivalent—that is, diabetes confers a 10-year CHD risk equal to that of persons with existing CHD, or >20%—and recommends treating patients with type 2 diabetes the same as patients with established CHD. Similarly, recently published guidelines of the Third Joint Task Force of European and other Societies recommend the same total-cholesterol and LDL-cholesterol goals in patients with diabetes as for those with established cardiovascular disease. However, in recognition of the fact that the absolute risk for CHD in patients with diabetes appears to vary in different populations, the PRO-CAM risk-assessment chart counts diabetes as one of several risk factors that are incorporated into absolute risk assessment.

Metabolic Syndrome

The metabolic syndrome encompasses a range of cardiovascular risk factors. Slightly different definitions of the metabolic syndrome have been proposed by a number of organizations, including the NCEP and the World Health Organization. According to NCEP criteria, a diagnosis requires the concomitant presence of 3 or more of the following biological and physiological abnormalities:

- elevated triglycerides (≥150 mg/dL [≥1.7 mmol/L])
- low HDL cholesterol (men <40 mg/dL [<1.03 mmol/L]; women <50 mg/dL [<1.29 mmol/L])
- impaired fasting glucose (≥110 mg/dL)
- high blood pressure (≥130/85 mm Hg), and
- increased waist circumference (men ≥40 inches [≥102 cm]; women ≥35 inches [≥88 cm])

Regardless of which definition is used, the presence of the metabolic syndrome is believed to increase a patient’s risk for CHD at any level of LDL cholesterol.

Analyses from the Framingham Heart Study have shown that men and women who have high triglyceride levels (>150 mg/dL [>1.7 mmol/L]) and a low level of HDL cholesterol (<40 mg/dL [<1.03 mmol/L]) are characterized by a significantly increased cardiovascular risk. The high triglyceride/low HDL cholesterol phenotype is a hallmark of the metabolic syndrome. The metabolic syndrome is closely associated with insulin resistance and is highly associated with the risk of CHD. It has a greater impact on the incidence of CHD in women than in men. Leptin is a protein that plays a role in fat metabolism and closely correlates with insulin resistance and other markers of the metabolic syndrome, independent of total adiposity. Elevated leptin levels have been proposed as an independent risk factor for CHD in a large prospective study (West of Scotland Coronary Prevention Study [WOSCOPS]).

Lipid Risk Factors

Triglycerides

Although the impact of hypertriglyceridemia on CHD risk has long been a matter of intense debate, considerable
evidence supporting the prognostic value of this lipid fraction has been gained during the last decade. A meta-analysis of 17 prospective trials found hypertriglyceridemia to be an independent risk factor for cardiovascular disease.\textsuperscript{10} Data from the PROCAM study showed a significant relation between hypertriglyceridemia and CHD risk, independent of LDL-cholesterol and/or HDL-cholesterol levels.\textsuperscript{11} Triglycerides were also associated with CHD risk in the Caerphilly Heart Disease Study (CHDS), again independent of total cholesterol and HDL-cholesterol levels.\textsuperscript{12} At the same time, no prospective, controlled trials have demonstrated the benefits of triglyceride-lowering agents also affect other lipid and lipoprotein concentrations.

Although the PROCAM scoring scheme includes triglycerides as a major independent risk factor, the NCEP ATP III guidelines do not. However, the ATP III gives increased emphasis to elevated triglycerides compared with ATP II, with triglycerides regarded as a marker for increases in triglyceride-rich remnant lipoproteins and for other lipid and nonlipid risk factors in the metabolic syndrome. In addition, the ATP III guidelines have lowered cut points for the classification of triglyceride levels, with normal triglycerides now defined as $<150$ mg/dL ($<1.7$ mmol/L).

**Triglyceride-Rich Remnant Lipoproteins**

Triglyceride-rich lipoproteins comprise a great variety of nascent and metabolically modified lipoprotein particles. The capacity to enter the subintestinal area of the vasculature is inversely related to the size of the lipid particle. Whereas chylomicrons and large very-low-density lipoprotein (VLDL) particles are unable to pass through the endothelial layer, smaller VLDL, intermediate-density lipoprotein (IDL), and LDL particles can enter the subintestinal space. Data show a direct relation between (small) VLDL/IDL and atherogenesis. Remnant lipoproteins derived from chylomicrons and/or VLDL (for example, lipoprotein subclasses LP-B:C, LP-B:C:E, and LP-A-II:B:C:D:E) have also been shown to promote atherogenesis. Interestingly, in the Monitored Atherosclerosis Regression Study (MARS), triglyceride-rich lipoproteins were particularly correlated with the rate of progression of mild/moderate ($<50\%$) stenosis, rather than severe ($\geq50\%$ stenosis) coronary lesions.\textsuperscript{13} In a prospective case-control study (Etude Cas Temoins sur Infarctus du Myocarde [ECTIM]), particles containing apolipoprotein (apo) C-III were increased in survivors of an MI compared with controls.\textsuperscript{14} VLDL-cholesterol, VLDL-triglyceride, VLDL-apo B, apo C-III, apo E in VLDL+LDL, and apo E in HDL all predicted subsequent coronary events in the Cholesterol and Recurrent Events (CARE) trial.\textsuperscript{15} VLDL particles and apo C-III in VLDL and LDL more reliably predicted CHD risk than did plasma triglycerides.\textsuperscript{15} Remnant-like particles (RLPs) can be isolated from the plasma by an antibody-based assay. In the Framingham Offspring Study, both RLP-cholesterol and RLP-triglycerides were significantly increased in women with diabetes ($P<0.0001$) and men ($P<0.001$) compared with controls without diabetes.\textsuperscript{16}

**Lipoprotein(a)**

Lipoprotein(a) [Lp(a)] is formed by joining a lipoprotein that is structurally similar to LDL in protein and lipid composition to a carbohydrate-rich, hydrophilic protein called apo(a). Lp(a) particles contain apo(a) and apo B in a 1:1 molar ratio. Apo(a) contains a kringle domain and a carboxyl-terminal domain with 85\% amino acid identity with the plasminogen protease domain. The molecular mass of apo(a) protein varies from 187 kDa for an apo(a) that contains 12 kringle 4 domains, to 662 kDa for an apo(a) that contains 50 kringle 4 domains.

To date, no clinical trials have shown that lowering Lp(a) levels decreases CHD risk. However, almost all retrospective case-control studies have found a strong association between elevated Lp(a) levels and CHD. On the other hand, the results of prospective studies have been mixed, with 9 prospective studies reporting that Lp(a) is an independent risk factor for CHD and 4 other studies reaching the opposite conclusion. These inconsistencies may (in part) be related to a lack of standardization and the failure of some immunoassays to measure all apolipoprotein(a) isoforms. A recent meta-analysis of prospective studies indicated that plasma Lp(a) concentration is indeed an independent risk factor for CHD in both men and women.\textsuperscript{17} This conclusion was confirmed by the Prospective Epidemiological Study of Myocardial Infarction (PRIME),\textsuperscript{18} which included 9133 French and Northern Irish men, aged 50 to 59 years at entry, without manifest cardiovascular disease. In these subjects, Lp(a) was significantly related to CHD development and appeared to be a significant risk factor ($P<0.0006$) in the cohort as a whole (although the association was not statistically significant in the Belfast sample). More specifically, this study found that subjects with levels of Lp(a) in the highest quartile had more than 1.5 times the risk than subjects in the lowest quartile. Moreover, a Lp(a) level above 33 mg/dL and a high LDL cholesterol ($>163$ mg/dL [$>4.22$ mmol/L]) was associated with increased cardiovascular risk compared with Lp(a) levels below 33 mg/dL and low LDL cholesterol ($<121$ mg/dL [$<3.13$ mmol/L]; relative risk: 1.58 and 0.82, respectively). This study also showed that elevations in Lp(a) increased the risk for MI and angina pectoris, especially in men with a high LDL-cholesterol level.

**Homocysteine**

Homocysteine is formed during demethylation of methionine, whereas its degradation takes place via remethylation and/or transsulfuration. Impaired homocysteine metabolism has been implicated as a factor in atherosclerosis, cerebrovascular disease, and peripheral vascular disease. The causes of hyperhomocysteinemia include genetic causes (ie, thermo-labile methylentetrahydrofolate reductase, heterozygous cystathionine synthase), vitamin deficiency (folic acid, B$_{12}$, B$_{6}$), the use of certain medications, and impaired renal function. In addition, direct relationships between homocysteine and cigarette smoking, diabetes, obesity, and hypertension have been suggested. The exact mechanism by which higher homocysteine levels may translate into increased CHD and/or thrombotic risk remains speculative. Both direct toxic effects on endothelial cells, in part due to oxidative stress, as well as more indirect mechanisms have been postulated.

To date, more than 80 cross-sectional, case-control, and cohort studies have linked hyperhomocysteinemia with CHD.
risk. For example, in the Framingham Heart Study, a nested case-control study in 21,826 subjects in Tromso, Norway, and in women but not men enrolled in the Atherosclerosis Risk in Communities (ARIC) study, homocysteine levels were higher in adults with CHD. In the British Regional Heart Study, homocysteine levels were significantly ($P=0.004$) higher in patients with stroke. Most recently, data from a prospective cohort study in 17,361 persons corroborated the finding of increased homocysteine levels in subjects with preexisting cardiovascular disease. Moreover, the frequency of hospitalization for cardiovascular disease was correlated with baseline homocysteine levels, particularly in the oldest age group (hospitalization-rate ratio per 5 μmol/L increase in homocysteine: 1.29 versus 1.10; probability value for interaction, 0.02). Homocysteine was measured in healthy French participants in the Supplementation with Antioxidant Vitamins and Minerals Study, which is investigating the effects of antioxidant supplementation on chronic diseases. This study suggested that to control homocysteine, decreasing coffee and alcohol consumption may be important in women, whereas increasing physical activity, dietary fiber, and folate intake may be important in men.

Currently, the impact of treating hyperhomocysteinemia in subjects at increased cardiovascular risk is being evaluated in several prospective studies, data from which will become available over the next 2 to 4 years. Awaiting these results, recent analyses have emphasized the potential impact of “regression dilution” on the potential outcome. This refers to within-person variability in plasma homocysteine measurements, which dilute the association of homocysteine with CHD risk. Regression dilution ratios (RDR) for homocysteine were calculated using replicate homocysteine measurements obtained after 3, 6, and 8 years from the Rotterdam, Hordaland, and Framingham studies, respectively, and after 3, 6, 9, and 12 years from the United Kingdom Prospective Diabetes Study (UKPDS). Using linear regression analysis for the population-based studies, these results show an RDR of 0.83 at 2 years, 0.71 at 6 years, and 0.53 at 12 years. Extrapolation of these findings to the ongoing prospective studies suggests that these studies may underestimate the relative risks for CHD associated with homocysteine by 20% after 2 years and 50% after 10 years.

Thrombogenic/Hemostatic Factors
The incidence of CHD is higher in Northern Ireland than in France, for reasons that have not been adequately explained. The associations of plasma fibrinogen concentration and factor VII activity with the incidence of CHD were evaluated in the PRIME study. Baseline mean levels of fibrinogen were significantly higher in Belfast than in France, and they were higher in both countries in participants who experienced coronary events compared with those who did not. The age-adjusted relative risk of CHD associated with an increase of one standard deviation in fibrinogen level was 1.56 in the whole cohort and remained significant ($P<0.0001$) after adjustment for other cardiovascular risk factors. There was no clear geographical variation in factor VII, and no significant association was observed between factor VII levels and the risk of coronary events. Classic risk factors explained 25% of the excess risk of CHD in Belfast compared with France, whereas fibrinogen alone accounted for 30%. These findings add to the epidemiological evidence that elevated plasma fibrinogen is a potential risk factor for CHD. Other thrombogenic/hemostatic factors that have been investigated for their potential role in atherogenesis and/or thrombosis include von Willebrand factor and plasminogen activator inhibitor-1.

High-Sensitivity C-Reactive Protein and Other Inflammatory Markers
Atherosclerosis represents a chronic inflammatory state, and inflammatory parameters (eg, interleukin-6, tumor necrosis factor-α) have predictive value for future cardiovascular disease. Data have accumulated to show that C-reactive protein has additive value for predicting CHD risk on top of traditional risk factors. In prospective studies, healthy men and women with increased baseline levels of high-sensitivity C-reactive protein (hs-CRP) were at increased risk for future CHD. Hs-CRP is associated with subclinical epicardial coronary calcification in men and women and is significantly ($P\leq0.005$) elevated in patients dying suddenly with severe coronary artery disease, both with and without acute coronary thrombosis; it also correlates with immunohistochemical staining intensity and numbers of thin cap atheroma. Moreover, one study reported that hs-CRP is a risk factor independent of traditional risk factors such as total cholesterol, HDL cholesterol, age, smoking, body mass index, and blood pressure. High plasma levels of hs-CRP may be associated with a higher incidence of late adverse events after successful coronary stenting. Elevated hs-CRP constitutes an independent predictor of advanced carotid plaques in dyslipidemic subjects, and early-onset carotid atherosclerosis is associated with increased intima-media thickness and elevated serum levels of inflammatory markers. A graded association between hs-CRP and carotid atherosclerosis has been demonstrated in women but not in men. Recently, the American Heart Association has endorsed optional use of hs-CRP to guide physicians in identifying patients without known cardiovascular disease who may be at higher risk than estimated by major risk factors alone. This additional information might, in turn, guide the clinician in considering further evaluation (eg, imaging, exercise testing) or therapy (eg, lipid-lowering, antiplatelet, or cardioprotective drugs).

Matrix metalloproteinase 9 concentration has been identified as a novel predictor of cardiovascular mortality in patients with coronary artery disease. Whether it provides independent prognostic information compared with other inflammatory markers requires additional assessment. Elevated serum levels of interleukin-10 are associated with a more favorable prognosis in patients with acute coronary syndromes and elevated CRP levels. These data demonstrate the importance of the balance between proinflammatory and antiinflammatory markers as a major determinant of patients’ outcome in acute coronary syndromes.

Conclusion
Atherosclerosis is a multifactorial disease. The impact of traditional risk factors such as age, sex, elevated blood pressure, smoking, high levels of LDL cholesterol, and low
levels of HDL cholesterol on CHD risk has long been demonstrated beyond any doubt. More recent analyses show that increased triglyceride levels are also associated with increased CHD risk. In particular, triglyceride-rich lipoprotein remnants associated with apo C-III appear to have a major impact on risk. As promising novel risk factors, the additive value of homocysteine and hs-CRP for assessment of CHD risk is being evaluated in ongoing prospective studies. The combination of traditional risk factors and emerging risk factors is expected to facilitate the assessment of patients’ global risk, thereby allowing optimal use of diagnostic and therapeutic efforts in high-risk subjects.

References
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