Emerging Risk Factors for Atherosclerotic Vascular Disease
A Critical Review of the Evidence

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Cardiovascular disease (CVD) is the leading cause of death and disability in developed nations and is increasing rapidly in the developing world. By the year 2020, it is estimated that CVD will surpass infectious diseases as the world's leading cause of death and disability. Atherosclerotic vascular disease (ASVD), which encompasses coronary heart disease, cerebrovascular disease, and peripheral arterial disease, is responsible for the majority of cases of CVD in both developing and developed countries. Fortunately, major progress in the diagnosis, prevention, and treatment of atherosclerosis has been made in the past 3 decades.

One of the most important advances in medicine has been the identification of the major risk factors for CVD, which arose from large prospective cohort studies such as the Framingham Heart Study and the Seven Countries Study. The major modifiable risk factors include elevated blood pressure, dyslipidemia, smoking, and diabetes mellitus. A substantial body of evidence now supports reducing these factors to reduce morbidity and mortality associated with ASVD. Indeed, screening for and treating these conditions forms the basis of many published guidelines of risk assessment and reduction strategies.

It is apparent, however, that a substantial proportion of cardiovascular events occurs in individuals without...
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Box. Novel Risk Factors for Atherosclerotic Vascular Disease

Inflammatory Markers
C-reactive protein
Interleukins (eg, IL-6)
Serum amyloid A
Vascular and cellular adhesion molecules
Soluble CD40 ligand
Leukocyte count

Hemostasis/Thrombosis Markers
Fibrinogen
von Willebrand factor antigen
Plasminogen activator inhibitor 1 (PAI-1)
Tissue-plasminogen activator
Factors V, VII, and VIII
D-dimer
Fibrinopeptide A
Prothrombin fragment 1 + 2

Platelet-Related Factors
Platelet aggregation
Platelet activity
Platelet size and volume

Lipid-Related Factors
Small dense low-density lipoprotein (LDL)
Lipoprotein(a)
Remnant lipoproteins
Apolipoproteins A1 and B
High-density lipoprotein subtypes
Oxidized LDL

Other Factors
Homocysteine
Lipoprotein-associated phospholipase A(2)
Microalbuminuria
Insulin resistance
PAI-1 genotype
Angiotensin-converting enzyme genotype
Apolipoprotein E (apoE)
Infectious agents: Cytomegalovirus, Chlamydia pneumonia, Helicobacter pylori, Herpes simplex virus

Psychosocial factors

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these established risk factors. The reasons for this are multifactorial; in particular, there is evidence that even modest elevations of blood pressure, cholesterol, and glucose levels combine to place individuals at risk for CVD. Despite the fact that most cardiovascular events are explained by conventional risk factors, the search for additional etiologic agents continues. Moreover, while the population-attributable risk of the major vascular risk factors is substantial, it is often difficult to distinguish those individuals with a moderate baseline risk who might benefit from aggressive risk reduction strategies. Therefore, additional tests to assist in the prediction of risk in these individuals may be warranted.

In recent years, a number of new candidate risk factors or markers have been proposed as significant predictors of atherosclerosis and its complications (Box). This review will highlight 4 important emerging risk predictors: C-reactive protein (CRP), lipoprotein(a) [Lp(a)], fibrinogen, and homocysteine. These 4 risk predictors were selected because there is substantial evidence on their predictive abilities, there is a genetic basis for premature disease, modifying treatments are available, and/or these factors are the subject of ongoing or completed clinical trials (Table).

METHODS

Using the terms atherosclerosis, cardiovascular disease, risk factors, prevention, screening, C-reactive protein, lipoprotein(a), fibrinogen, and homocysteine, we searched the MEDLINE database from January 1990 to January 2003. Conference proceedings, abstract booklets, bibliographies of pertinent articles and books, and personal files were hand searched to identify additional articles. We selected original investigations and reviews of the epidemiology of atherosclerosis and the associations of conventional and novel risk factors with vascular risk. A diverse array of studies was examined, including controlled trials, prospective cohort studies, systematic overviews, case-control, cross-sectional, and mechanistic studies. Data extraction was performed by one of the authors (D. G. H.). For this narrative review, we focused our attention on assessing the clinical significance and additive predictive value of 4 candidate risk factors in comparison with established and validated risk prediction tools, such as the Framingham Risk Score (FRS).
The predictive abilities of CRP seem to extend to patients with preexisting vascular disease as well. A number of prospective studies have demonstrated that CRP predicts recurrent events and/or increased mortality in patients with ischemic stroke, acute coronary syndromes, chronic stable angina, and peripheral vascular disease. An elevated CRP level before percutaneous coronary intervention also portends a worse prognosis, as it does among patients undergoing coronary artery bypass grafting. C-reactive protein is correlated with the presence of abdominal obesity and a raised level predicts the risk of developing type 2 diabetes. The predictive value of CRP is additive to that of several surrogate markers of atherosclerosis, including carotid intimal medial thickness, quantitative coronary calcium scoring, and the presence of the metabolic syndrome. Whether or not CRP is a marker or mediator of inflammation is unclear.

There is evidence that CRP may play a direct role in the pathogenesis of atherosclerosis. The protein is markedly up-regulated in atheromatous plaques, where it may promote low-density lipoprotein (LDL) cholesterol uptake by macrophages, a key step in atherogenesis. C-reactive protein may also induce the expression of intercellular adhesion molecules by endothelial cells, thereby facilitating the recruitment of circulating monocytes to plaque sites, and can bind to and activate complement in serum. These effects appear to be mediated through CRP-induced secretion of endothelin 1 and IL-6.

However, the utility of CRP as a tool in global risk assessment has some important limitations. These include CRP’s poor specificity in the setting of coexisting inflammatory states (eg, rheumatoid arthritis, chronic pulmonary disease, and infections) and minimal data from nonwhite populations. In addition, CRP is strongly correlated with other cardiovascular risk factors such as fibrinogen; in studies examining CRP’s incremental predictive value, its independence from fibrinogen has not been demonstrated. For example, in patients with established vascular disease, fibrinogen, but not CRP, was a significant independent predictor of recurrent cardiovascular events after adjustment for conventional risk factors. We are aware of only one study that formally compared the incremental predictive value of CRP added to that of an established risk prediction model (the FRS). In this latter analysis, the authors found that CRP remained a predictor of cardiovascular risk after adjustment for the FRS, although there was attenuation in the magnitude of risk after adjustment was performed. The incremental value of CRP in addition to the Framingham factors may have been overestimated given that the model was not adjusted for body mass index, abdominal fat, or physical activity levels—3 factors that are significant correlates of CRP. Further studies are required to clarify these issues.

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Table. Characteristics of 4 Emerging Risk Factors for Atherosclerotic Vascular Disease

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Chemical Properties</th>
<th>Site of Synthesis</th>
<th>Biological Function</th>
<th>Modulating Factors</th>
<th>Risk Prediction†</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-reactive protein</td>
<td>Pentameric protein</td>
<td>Liver</td>
<td>Involved in innate immunity Acute-phase reactant</td>
<td>Inflammation Weight loss Smoking HRT (estrogen and progesterone)</td>
<td>Strong Moderate</td>
</tr>
<tr>
<td>Lipoprotein(a)</td>
<td>LDL-like particle</td>
<td>Liver</td>
<td>Acute-phase reactant</td>
<td>Inflammation Genetics</td>
<td>Moderate Unknown</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>Serum glycoprotein</td>
<td>Liver</td>
<td>Cleared by thrombin to form fibrin Acute-phase reactant</td>
<td>Inflammation Smoking Age</td>
<td>Moderate Minimal</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>Amino acid byproduct Converted to cysteine and/or methionine</td>
<td>Intracellular</td>
<td>No known biological role</td>
<td>Renal failure Hypothyroidism Folate/B12 deficiency</td>
<td>Moderate Minimal</td>
</tr>
</tbody>
</table>

Abbreviations: HRT, hormone replacement therapy; LDL, low-density lipoprotein.

†Overall risk prediction: minimal, there is limited information (ie, case-control/cross-sectional and/or retrospective data) that the risk factor is an independent predictor of cardiovascular risk; moderate, there is moderate information (long-term, prospective, longitudinal studies) that the risk factor is an independent predictor of cardiovascular risk; strong, there is strong and consistent information (from multiple prospective studies) that the risk factor is an independent predictor of cardiovascular risk. Additive risk prediction refers to the incremental or additive predictive value of a risk factor, over that of a validated risk prediction model, such as the Framingham Risk Score.
thermore, in this large prospective cohort study, covariates such as blood pressure, smoking, and diabetes were self-reported rather than measured, which may underestimate the impact of these factors on vascular risk.66

Retrospective subgroup analyses have raised the possibility that statins and aspirin (which lower CRP levels) may lead to reduced cardiovascular events even among patients without overt hyperlipidemia.47,48 In addition, some investigators advocate that a strategy of CRP screening to target statin therapy for the primary prevention of CVD among patients without overt hyperlipidemia could be cost-effective.49 However, to date, no clinical trials have prospectively demonstrated that targeting patients with elevated CRP lowers vascular event rates, or whether interventions aimed at lowering CRP translates into reduced vascular risk, although several are ongoing.50

Lipoprotein(a)

Lipoprotein(a) is an LDL-like particle in which an apolipoprotein(a) [apo(a)] moiety is linked via a disulfide bond to apolipoprotein B-100.51 Concentrations of Lp(a) are largely under genetic control and vary substantially between individuals depending on the size of the apo(a) isoform present; conversely, Lp(a) levels vary little with diet or exercise, unlike other lipoproteins such as LDL and high-density lipoprotein (HDL).52 The wide range of Lp(a) in plasma within a population is due in large part to a variable number of plasminogen-like kringle IV repeats, and an inverse correlation between the number of kringle IV type 2 repeats in the apo(a) gene and Lp(a) plasma concentration exists.53 The biological function of Lp(a) is still unclear, but there is strong evidence that its phylogenetic role may have been to respond to tissue injury and vascular lesions, prevent infectious pathogens from invading cells, and promote wound healing.54

Lipoprotein(a) is an acute-phase reactant, more than doubling in concentration in response to the proinflammatory cytokine IL-6.55 Lipoprotein(a) binds avidly to endothelial cells, macrophages, fibroblasts, and platelets, as well as to the subendothelial matrix; there, it may promote proliferation of vascular smooth muscle cells and chemotaxis of human monocytes.56-58 However, its most important putative role in atherothrombosis may be to inhibit clot fibrinolysis at sites of tissue injury. By virtue of its unique structural homology to plasminogen, Lp(a) is thought to compete with plasminogen for binding to plasminogen receptors, fibrinogen, and fibrin.59 Lipoprotein(a) may also induce the production of plasminogen activator inhibitor 1 (the main inhibitor of the fibrinolytic system) and may inhibit the secretion of tissue-plasminogen activator by endothelial cells.60,61

By virtue of these properties, as well as its ability to deliver a rich source of cholesterol to sites of vascular injury (cholesterol represents almost 40% of its mass), Lp(a) has been postulated to be a highly atherothrombotic lipoprotein; the epidemiological evidence largely supports this hypothesis, albeit with several notable exceptions.62,63 A recent meta-analysis of 27 prospective studies with a mean follow-up of 10 years showed a combined risk ratio of 1.6 (95% CI, 1.4-1.8) for individuals in the top third of baseline Lp(a) concentrations compared with those in the bottom third.64 Adjustment for conventional risk factors did not diminish this association. Moreover, an elevated Lp(a) level may be particularly detrimental in the presence of high HDL levels, diabetes, low HDL levels, hypertension, hyperhomocysteinemia, or elevated fibrinogen concentrations.55-68

Nonetheless, the use of Lp(a) as a screening tool has some limitations. No universally accepted, standardized method of determination for Lp(a) exists, although recently, a working group of the International Federation of Clinical Chemistry demonstrated the inaccuracy of Lp(a) values determined by methods sensitive to apo(a) size and recommended the widespread implementation of a proposed reference ma-

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tween fibrinogen and ischemic stroke and peripheral vascular disease, again largely independent of the classic risk factors.

Several factors other than inflammation have been shown to modulate fibrinogen levels. Smoking and smoking cessation are associated with an increase or decrease, respectively, of approximately 0.15 g/L in plasma fibrinogen. Furthermore, there is a dose-response relationship between number of cigarettes smoked and fibrinogen level. Fibrinogen levels tend to be higher in patients with diabetes, hypertension, obesity, and those with sedentary lifestyles. Fibrates and niacin lower fibrinogen levels (in addition to lipid parameters), whereas statins and aspirin do not. A recently completed, randomized controlled trial examined the effects of bezafibrate (400 mg/d) on cardiovascular event rates in 1,568 patients with peripheral vascular disease. Despite a 13% reduction in fibrinogen (and a favorable effect on serum lipids, especially triglycerides), there was no reduction in the incidence of coronary heart disease events or stroke. Further clinical trials are necessary before it can be determined whether fibrinogen has a causal role in atherothrombosis or is merely a marker of the degree of vascular damage taking place. Finally, we found only one study that examined the additive yield of screening for fibrinogen in comparison with a validated scheme, such as the FRS. Although an elevated fibrinogen level remained independently predictive of cardiovascular events after adjustment for the FRS, the study entailed a high-risk secondary prevention cohort attending a vascular prevention clinic, and therefore, the possibility of selection bias and reverse causality cannot be excluded.

Homocysteine
Homocysteine is a highly reactive, sulfur-containing amino acid formed as a by-product of the metabolism of the essential amino acid methionine. Cells remetabolize homocysteine by a number of possible pathways involving several different enzymes; these enzymes variably use B vitamins as substrates or cofactors, namely folate, cobalamin (vitamin B12), and pyridoxine (vitamin B6).

In the 1960s and 1970s, 3 different inborn errors of homocysteine metabolism involving these enzymes were described (termed “homozygous homocystinurias”). Common to all 3 disorders are extremely high levels of homocysteine in the blood and urine of individuals homozygous for these mutations; half of affected individuals develop arterial or venous thrombosis by 30 years of age. This risk can be substantially ameliorated by the provision of high-dose B vitamins, which partially lower homocysteine levels back toward the normal range.

It has been postulated that mild to moderate elevations of homocysteine in the general population predispose to atherosclerosis in a manner akin to the classic risk factors. This is important because of the availability of an inexpensive, safe, and effective therapy for lowering homocysteine (B vitamins). Mechanistic studies have demonstrated that homocysteine may induce vascular damage by promoting platelet activation, oxidative stress, endothelial dysfunction, hypercoagulability, vascular smooth muscle cell proliferation, and endoplasmic reticulum stress. A common gene mutation encoding one of the enzymes that metabolizes homocysteine (5,10-methylenetetrahydrofolate reductase [MTHFR]) leads to moderate increases in homocysteine levels on the order of 25%, particularly in the presence of low folate intake. Homozygosity for this MTHFR variant (677C→T) is present in up to 15% of the Caucasian population, thus providing a natural experiment by which the relationships between the abnormal MTHFR genotype, higher homocysteine levels, and vascular disease can be discerned. Recently, 2 large meta-analyses of the MTHFR 677C→T polymorphism confirmed modest but statistically significant increases in the risk of ischemic heart disease in homozygotes for the mutant allele (TT) compared with wild-type homozygotes (CC), with summary ORs of 1.21 (95% CI, 1.06-1.39) and 1.16 (95% CI, 1.05-1.28), respectively.

Numerous observational studies have also reported on the association between homocysteine levels and vascular risk in both the general population and in those with preexisting vascular disease. A number of meta-analyses have appeared in recent years in an attempt to summarize the evidence. In general, prospective, longitudinal studies in healthy populations have offered substantially weaker support to the association between homocysteine and ASVD than retrospective case-control and cross-sectional studies. However, more recent meta-analyses that collected larger numbers of prospective studies and/or corrected for regression dilution bias (the intraindividual variability in homocysteine levels over follow-up) do show a significant association. We found only one study of the risk prediction capabilities of homocysteine measurement with reference to the FRS (which also examined fibrinogen). As mentioned previously, this study specifically focused on a high-risk population with existing CVD; furthermore, only overall mortality (and not cause-specific death) was reported in longitudinal analyses.

Whether homocysteine is causative in the pathogenesis of atherosclerosis, is related to other confounding cardiovascular risk factors, or is a marker of existing vascular disease will have to await the completion of a large, randomized controlled trials studying the effect of homocystine-lowering vitamins on cardiovascular end points. Although mandated cereal fortification with folic acid may affect the outcomes of clinical trials in North America, several studies are being conducted in regions of the world that have not mandated folate supplementation (eg, Australia and Europe).

One trial in patients undergoing coronary angioplasty has already been published. Schinzer et al randomized 205 patients following successful coro-
nary angioplasty to a 6-month course of folic acid, vitamin B₆, and vitamin B₁₂ or matching placebos, in double-blind fashion.¹¹₀ The risk of restenosis was reduced by 48% at 6 months in the group that received vitamins (relative risk, 0.52; 95% CI, 0.32-0.86), with a concomitant reduction in homocysteine of 35%. A 1-year follow-up of this study¹¹¹ with a larger group of patients (n=553) demonstrated these results were durable and persisted for a mean of 11 months (despite cessation of vitamins at 6 months). However, there was no reduction in myocardial infarction or death. It should also be noted that postangioplasty restenosis (which involves intimal hyperplasia) and atherosclerosis are different pathological processes, which therefore limits the generalizability of these results to other vascular patients.¹¹² Furthermore, recent data from another randomized controlled study of 626 patients treated with B-vitamin therapy following percutaneous coronary intervention found increased rates of restenosis and major adverse cardiac events in the vitamin treatment group after 6 months of follow-up.¹¹³

**COMMENT**

A number of critical questions must be answered before any candidate risk factor can be recommended for routine screening.¹¹⁴ First, there must be an available, standardized, reproducible, and accurate laboratory test that has been prospectively validated in the population to which it will be applied. For some of the factors discussed in this review, including Lp(a), this goal has not yet been achieved; for other factors (eg, CRP and homocysteine), precise and valid laboratory tests are now available.¹¹⁵,¹¹⁶

Second, it must be demonstrated that measurement of the candidate factor adds to the information obtained from existing approaches to cardiovascular risk stratification. A number of global risk assessment schemes are available to clinicians today, with one of the most validated being the FRS.³,¹¹⁷,¹¹⁸ As previously noted, we found only one study for each of CRP,¹¹⁴ homocysteine,⁸⁵ and fibrinogen,⁸⁵ and none for Lp(a), that directly assessed the additive yield of risk factor screening to the FRS or other commonly used methods of risk assessment.

Third, for each putative risk factor, there must be prospective controlled trials demonstrating that (1) targeting individuals with elevated levels of these risk factors for proven risk-reducing interventions offers advantages over current methods of targeting therapy (eg, by cholesterol, diabetes, and blood pressure screening); or (2) selectively and specifically reducing the risk factor reduces hard cardiovascular end points, such as mortality, nonfatal myocardial infarction, and stroke. For the risk markers reviewed here, we found only 2 clinical trials (both for homocysteine) meeting the latter criterion,¹¹⁰,¹¹¹ although we are aware that a number are in progress, particularly with respect to homocysteine and CRP.⁵⁰,¹¹⁹

Fourth, the diagnostic characteristics of any screening test are only applicable to the population from which they are derived. The sensitivity and specificity of a test may vary according to prevalence or spectrum of severity of disease in a given population.¹²⁰ It is therefore critical that promising data on novel risk factor screening in a secondary prevention population, for instance, not be generalized to apparently healthy, asymptomatic individuals with vascular risk factors, or vice versa, in the case of CRP. Furthermore, as each population may be heterogeneous with respect to patient characteristics, it is likely that no single level of a candidate risk factor can be derived that is adequate for all subgroups.

One of the key motivating forces for searching for novel risk factors for ASVD has been the claim that only 50% of the risk of atherosclerosis can be explained by the classic, established risk factors.¹²¹,¹²² However, there appears to be no solid evidence on which this claim is based. Long-term follow-up from the Multiple Risk Factor Intervention Trial (MRFIT), a cohort of some 356,222 persons screened for primary prevention in the United States, showed that 92% of coronary heart disease deaths could be explained by suboptimal levels of cholesterol, blood pressure, cigarette smoking, and diabetes.⁸ Furthermore, the explanatory power of the most recent set of equations from the Framingham study, which in large part address the same variables, is approximately 75% to 77%.¹ Even this figure is likely to be an underestimate of the true strength of the conventional risk factors, due to regression dilution bias, surrogate dilution effect, and misclassification error inherent in using arbitrary cutpoints for continuous risk factors.¹²¹,¹²³,¹²⁴ Therefore, the explanatory power of the classic risk factors for ASVD is likely to be much higher than has been previously assumed.

There are other clear advantages to using a global risk assessment strategy, rather than relying on the measurement of single risk factors, whether conventional or novel. This has been reviewed in detail by Pasternak, who cites a number of important benefits.¹²⁵ The use of global risk score (1) raises awareness that risk is continuous and graded and related to the overall burden of risk factors; (2) enables adjustment for the severity of individual component risk factors; (3) emphasizes that the clinician must approach the patient as a whole and not be excessively distracted by individual risk factors when multiple risk factors coexist; (4) promotes the use of risk-lowering strategies based on multiple components of an individual’s risk; and (5) serves as a motivating, instructive force for both patients and clinicians. Furthermore, it should be noted that among many populations previously studied, levels of the classic vascular risk factors are suboptimally recognized and treated, and thus more emphasis should be placed on screening for and treating the conventional ASVD risk factors, given the proven value of targeting and lowering them.⁹,¹²⁶

There are, however, several situations for which we can envision mea-
suring a panel of these 4 novel factors: (1) asymptomatic individuals with strong family histories of vascular disease in whom an excess of conventional risk factors is not apparent; (2) patients with premature vascular disease with no apparent explanatory factors; and (3) individuals with aggressive or recurrent vascular disease despite optimal management of all conventional risk factors (both by lifestyle and pharmacological means). However, it should be noted that evidence does not yet exist to support reducing these novel factors, yet the risk-benefit ratio could still be highly favorable (in some scenarios), given the innocuous nature of some therapies (eg, B vitamins) and the likelihood of further vascular events. With respect to CRP measurement, we agree with recent guidelines released by a joint panel of the American Heart Association and the Centers for Disease Control and Prevention that application of secondary prevention measures (either in the acute coronary syndrome setting or for long-term treatment of atherosclerosis) should not depend on CRP determination, since such patients are at heightened risk of future vascular events and should be targeted for aggressive treatment irrespective of CRP level.11

CONCLUSION

Despite support from epidemiological and basic science, more investigation is needed before measurement of CRP, Lp(a), fibrinogen, and homocysteine can be recommended for widespread use in primary or secondary prevention settings. The explanatory strength of the conventional risk factors has been underestimated, and perhaps in part because of this, detection and control of these important risks has been suboptimal across many populations. The focus of clinicians, policy makers, health care organizations, and patients must be on lowering the burden of proven, modifiable risk factors, including their upstream determinants (eg, obesity), if the burgeoning epidemic of CVD is to be altered.

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cytochrome P450, which is necessary for the metabolism of hormones and other molecules. Folic acid supplementation has been shown to lower homocysteine levels, which in turn can reduce the risk of cardiovascular disease.

However, the implications of homocysteine levels are complex and require further investigation. Further research is needed to determine the optimal levels of homocysteine and the long-term effects of supplements on cardiovascular health.