Abstract—There is great interest in moving beyond established risk factors to consider markers of inflammation for the prediction of initial cardiovascular disease events. Inflammatory markers such as leukocyte count, serum amyloid A, C-reactive protein, and vascular outcomes in individuals free of cardiovascular disease at baseline are the key markers that have been investigated in the population setting. A meta-analysis of 11 prospective studies in asymptomatic individuals compared people in the bottom third of the C-reactive protein distribution with those in the top tertile. The authors reported an odds ratio of 2.0 (95% CI 1.6 to 2.5) for coronary heart disease among people in the top tertile. These results are among the strongest assembled thus far to recommend incorporating newer biomarkers into coronary heart disease risk estimation algorithms. A variety of issues should be considered and conditions satisfied before vascular disease risk factors are adopted into regular use. The type of vascular event and the follow-up interval are important features because results for short-, intermediate-, and long-term intervals may yield different results. The factors under consideration should have been standardized, and characteristics such as the variability of the measurements, correlations with established factors, evidence from observational studies and clinical trials, type of effect (linear, nonlinear, dichotomous), improvement in overall prediction (discrimination), generalization of results (calibration), and cost can affect the utility. Each of these issues needs to be considered and the effects on relative, absolute, and population-attributable risks described. In particular, we need to (1) develop sound strategies for implementing new testing and (2) demonstrate the benefit of testing by using the current foundation of prior probabilities developed from already-published risk factor assessments based on large population studies. (Circulation. 2004;110:e568-e571.)

Key Words: AHA Scientific Statements • inflammation • cardiovascular diseases • homocysteine • C-reactive protein

Vascular disease is caused by many factors acting in concert. Several processes foster an atherosclerotic milieu within the coronary arteries, and clinical events are associated with temporary or permanent occlusions of these vessels. Research generally has demonstrated that lipids and blood pressure are critical ingredients that promote the long-term development of atherosclerosis, that cigarette smoking acts more as a precipitating factor, and that diabetes mellitus effects include several pathophysiological mechanisms. Each of these factors is used to estimate coronary heart disease (CHD) risk, but inflammatory and hematologic markers currently are not included in the roster of factors that are used to predict vascular disease risk in the population setting. We have a smaller body of data about the risks of vascular disease related to many of the newer inflammatory factors that predict vascular disease risk, but we do have information on several markers, including leukocyte count, fibrinogen level, homocysteine, and C-reactive protein (CRP). The authors of this paper have been acknowledged.
present article reviews some of the published evidence for these markers and vascular disease risk and provides a template for criteria that newer factors should fulfill before they can become effectively incorporated into risk-prediction strategies.

**Hematologic Factors**

Higher leukocyte count was shown to be associated with a greater cardiovascular risk >20 years ago, but the utility of this factor has suffered from many limitations. Leukocyte counts needed to be determined on fresh specimens, and current cigarette smoking was associated with increased leukocyte counts, limiting the utility of the test results. In addition, higher leukocyte counts were not specific for the augmentation of vascular disease risk, as the counts were known to be related to a variety of other conditions.\(^5\) The overall estimate of the effect of leukocyte count on CHD risk from a meta-analysis that compared the top third with the bottom third of the distribution was 1.4 (95% CI 1.3 to 1.5) in a meta-analysis of 7 studies by Danesh and colleagues.\(^6\)

Starting in the 1970s, elevated fibrinogen levels were shown to be a major independent risk factor for various heart disease and stroke outcomes in several population studies (Figure 1).\(^7,8\) Cardiovascular disease, CHD, and all-cause mortality were all increased in patients of both sexes who had higher fibrinogen concentrations, and this excess mortality persisted after adjusting for the standard risk factors. Higher fibrinogen levels enhanced the CHD risk of patients with hypertension, cigarette smokers, and people with diabetes. In Danesh and colleagues’ early meta-analysis of 18 studies they compared the top third of the distribution with the bottom third, and they found higher fibrinogen to be related to a relative risk of 1.9 (95% CI 1.3 to 1.5) in a meta-analysis of 7 studies by Danesh and colleagues.\(^6\)

**Figure 1.** Relative risk of vascular disease outcomes according to study-specific tertile of fibrinogen count in primary occurrence studies. Adapted with permission from Danesh et al.\(^6\)

**C-reactive protein and CHD**

Several meta-analyses have highlighted the promise of CRP as a factor that may be predictive of initial coronary disease events (Figure 2).\(^6,15\) It was estimated that the top third of the CRP distribution is related to a relative risk of 1.9 (95% CI 1.5 to 2.3) risk for CHD relative to the bottom third of the CRP distribution. An update in 2004 of the effects of CRP tempered the previous estimates, and the authors of a meta-analysis estimated that the top third of the CRP distribution was related to a relative risk of 1.45 (95% CI 1.25 to 1.68) for CHD when comparing the top third with the bottom third of the CRP distribution, after adjusting for other risk factors.\(^16\) The reviewers ascribed the moderating effects to newer, larger studies that have shown less consistent effects of CRP on vascular disease risk. Counterbalancing that sentiment, the top third of the CRP distribution may not be the best level to use for comparisons and absolute levels in the 3 to 5 mg/L range may be preferable.\(^17\) Evidence has linked higher CRP levels to various risk factors such as obesity,\(^18\) cigarette smoking,\(^19\) and estrogen use\(^20\) and to vascular outcomes, including cerebrovascular disease\(^21,22\) and peripheral vascular disease.\(^11\) Less convincing evidence has been published with regard to higher serum amyloid A protein and greater risk of vascular disease. The reports have been smaller and moderately inconclusive. Danesh et al\(^23\) and Ridker and coworkers\(^24\) have reported that higher serum amyloid A levels are also related to greater CHD risk, but further research is needed in this area.

**Homocysteine**

Cross-sectional, case-control, and prospective studies have linked increased levels of homocysteine to greater risk for cardiovascular disease,\(^11\) and the more recently developed ultrasensitive assays suggest improvement in coronary risk assessment over and above the use of conventional risk factors.\(^10–14\) Several meta-analyses have highlighted the promise of CRP as a factor that may be predictive of initial coronary disease events (Figure 2).\(^6,15\) It was estimated that the top third of the CRP distribution was related to a relative risk of 1.9 (95% CI 1.5 to 2.3) risk for CHD relative to the bottom third of the CRP distribution. An update in 2004 of the effects of CRP tempered the previous estimates, and the authors of a meta-analysis estimated that the top third of the CRP distribution was related to a relative risk of 1.45 (95% CI 1.25 to 1.68) for CHD when comparing the top third with the bottom third of the CRP distribution, after adjusting for other risk factors.\(^16\) The reviewers ascribed the moderating effects to newer, larger studies that have shown less consistent effects of CRP on vascular disease risk. Counterbalancing that sentiment, the top third of the CRP distribution may not be the best level to use for comparisons and absolute levels in the 3 to 5 mg/L range may be preferable.\(^17\) Evidence has linked higher CRP levels to various risk factors such as obesity,\(^18\) cigarette smoking,\(^19\) and estrogen use\(^20\) and to vascular outcomes, including cerebrovascular disease\(^21,22\) and peripheral vascular disease.\(^11\) Less convincing evidence has been published with regard to higher serum amyloid A protein and greater risk of vascular disease. The reports have been smaller and moderately inconclusive. Danesh et al\(^23\) and Ridker and coworkers\(^24\) have reported that higher serum amyloid A levels are also related to greater CHD risk, but further research is needed in this area.
not predictive of subsequent vascular events.\textsuperscript{15,26,28,29} Associations with a greater risk of coronary artery disease, carotid stenosis, stroke, and dementia have been demonstrated in participants in the Framingham Heart Study.\textsuperscript{30–33} Elevated homocysteine levels may be accompanied by decreased blood levels and intake of folate, vitamin B\textsubscript{6}, or vitamin B\textsubscript{12}.\textsuperscript{4} These vitamins are important cofactors in the metabolism of homocysteine. Borderline deficiencies may be relatively common, especially in older adults. Greater intake of these vitamins in the diet, via supplements in the form of multivitamins or through fortification of foods, may lead to less vitamin deficiency and to a decrease in the prevalence of elevated homocysteine levels.\textsuperscript{4,34} Fortification of the food supply with folate in the United States was announced in early 1996 and was enacted before January 1, 1998. Analyses of homocysteine and folate levels before and after fortification have demonstrated a dramatic decline in the prevalence of low folate levels, a reduction in the prevalence of elevated homocysteine from \( \frac{1}{10} \) to \( \frac{1}{10} \) mol/L, and a modest decrease in mean homocysteine levels from \( \frac{1}{10} \) to \( \frac{1}{10} \) \( \mu \text{mol/L} \).\textsuperscript{35} Concern has been expressed that case-control investigations with stronger findings have been more likely to reach publication and that the effect of homocysteine on vascular disease risk in prospective studies and in the folate-fortification era may be less than previously estimated.\textsuperscript{28}

Incorporation of New Risk Factors Into Prediction of CHD

New factors associated with increased risk for CHD arouse scientific interest and suggest that we may be able to improve the identification of individuals at risk for CHD. It is important that such new factors be biologically plausible, measurable, and repeatable. Furthermore, it is advantageous if the relation to disease is strong and graded and if treatment effects are demonstrable. Measurement issues include accuracy and precision for the factor in the laboratory and evidence of low or modest variability in the clinical setting. If the laboratory or biological variability is large, then the utility of the measurement for predictive purposes is seriously reduced. Many years of experience and standardization of measurements are available for factors such as blood cholesterol. For example, the analytical variability of cholesterol determinations is almost always \( <3\% \) in the modern era with standardized laboratory procedures. Biological sources of error, such as posture, season, fasting status, and intercurrent illness, in addition to other factors, also may be important, leading to a total variability that is usually \( <6\% \).\textsuperscript{36,37}

New risk factors may provide clues to pathogenesis and in some instances may improve our ability to predict disease. The ability to predict new CHD events should be demonstrated after consideration of the core set of factors that are currently available including age, sex, blood pressure, total cholesterol or LDL cholesterol (LDL-C), HDL-C, smoking, and type 2 diabetes mellitus. This criterion is often not met in new investigations, and considerable experience, relatively large data sets, and follow-up may be necessary to ensure that new factors such as inflammatory markers provide utility to predict vascular disease risk.

It would be advantageous if most of the concepts that were discussed in the preceding paragraphs were satisfied while prediction equations were developed to incorporate newer factors into CHD risk estimation equations. Many candidate factors are highly correlated with existing prediction variables, and these characteristics are important. For instance, once total cholesterol (or LDL-C) and HDL-C are included in a prediction equation, the added utility found for triglyceride information, used as a crude variable or after mathematical transformations, is relatively small.\textsuperscript{38}

Strategies have been developed for the use of newer diagnostic tests, and Figure 4 demonstrates this concept with a diagram. The abscissa and the ordinate represent prior probability and posterior probability of disease. If a screening test is performed and a second test is applied afterward but the probability of disease did not change, then the result is a line of unity.\textsuperscript{39,40} Such results occur rarely, and an “envelope” around the estimation line is the typical result. Positive results with a second test, shown by arrows pointing up, lead to an increased risk of disease, and the posterior probability is greater than the prior probability. Conversely, negative results with a second test, denoted by arrows pointing down,
will reduce the chance of disease, and the posterior probability is less than the initial probability of disease.

The utility of the newer inflammatory markers to improve risk estimation should be tested across a wide range of prior probabilities of CHD. The appropriate data sets to test are the populations to which we wish to apply the test—namely, across several age groups, in both sexes, and for different ethnic groups in population samples free of CHD at baseline. This approach will help to define not only the utility of “positive” tests but also the potential use of “negative” tests that may be related to the reduction in CHD risk because of new information that has not been contained in existing estimators of CHD risk.

References

CDC/AHA Workshop on Markers of Inflammation and Cardiovascular Disease: Application to Clinical and Public Health Practice: Ability of Inflammatory Markers to Predict Disease in Asymptomatic Patients: A Background Paper

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