

Novel Risk Factors for Atherosclerosis

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In the past several years, evidence has accumulated that factors other than conventional risk factors may contribute to the development of atherosclerosis. Conventional risk factors predict less than one half of future cardiovascular events. Furthermore, conventional risk factors may not have the same causal effect in different ethnic groups in whom novel risk factors may have a role. These newer risk factors for atherosclerosis include homocysteine, fibrinogen, impaired fibrinolysis, increased platelet reactivity, hypercoagulability, lipoprotein(a), small dense low-density lipoprotein cholesterol, and inflammatory-infectious markers. Identification of other markers associated with an increased risk of atherosclerotic vascular disease may allow better insight into the pathobiology of

atherosclerosis and facilitate the development of preventive and therapeutic measures. In this review, we discuss the evidence associating these factors in the pathogenesis of atherosclerosis, the mechanism of risk, and the clinical implications of this knowledge.

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CAD = coronary artery disease; CRP = C-reactive protein; ECAT = European Concerted Action on Thrombosis and Disabilities; HDL-C = high-density lipoprotein cholesterol; ICAM-1 = intercellular adhesion molecule 1; LDL-C = low-density lipoprotein cholesterol; Lp(a) = lipoprotein(a); MI = myocardial infarction; PAI-1 = plasminogen activator inhibitor type 1; tPA = tissue-type plasminogen activator

Many patients develop atherosclerosis in the absence of conventional risk factors. Studies have shown that conventional risk factors predict less than one half of future cardiovascular events.^{1,2} Furthermore, conventional risk factors may not have the same causal effect in different ethnic groups in whom other novel risk factors may have a role. For example, in South Asians, insulin resistance, lipoprotein(a) [Lp(a)], unidentified genetic-environmental interactions, low birth weight, and psychosocial factors may be important in the causation of atherosclerosis.³ Lack of an association between high prevalence of diabetes and coronary artery disease (CAD) in groups such as Afro-Caribbeans⁴ and low rates of CAD in Chinese and Japanese despite high smoking rates⁵ indicate that conventional risk factors may not have the same causal effect in different ethnic groups. In the past several years, evidence has accumulated that factors other than conventional risk factors may contribute to the development of atherosclerosis (Table 1). In this review, we focus on how these factors might be involved in the pathogenesis of atherosclerosis and the clinical implications of this knowledge.

HOMOCYSTEINE

Considerable evidence has accumulated to implicate increased plasma homocysteine levels as a risk factor for

atherosclerotic vascular disease.⁶ Homocysteine is a thiol-containing amino acid intermediate formed during the metabolism of methionine, an essential amino acid. In healthy persons, plasma homocysteine levels are between 5 and 15 μmol/L in the fasting state.⁷ Increased homocysteine plasma levels have been associated with aging,⁸ menopause,⁹ chronic renal insufficiency,¹⁰ low plasma levels of vitamin cofactors (B₆, B₁₂, and folate),¹¹ and cardiac transplantation.¹² Genetic abnormalities in homocysteine metabolism also may lead to increased levels. Homocysteine levels after a loading dose of methionine may classify an additional 27% to 40% of patients with CAD as having hyperhomocysteinemia.^{13,14} Determining a fasting homocysteine level is a simple cost-effective test to screen for hyperhomocysteinemia. The newer assays that are available for measuring homocysteine have increased sensitivity and specificity and are less expensive than the previously used high-pressure liquid chromatography method.

A meta-analysis of 27 studies indicated that increased homocysteine levels (>15 μmol/L) are associated with an increased risk of CAD, peripheral arterial disease, stroke, and venous thromboembolism.¹⁵ An increment of 5 μmol/L in the homocysteine level increases CAD risk as much as a 20-mg/dL increase in total cholesterol.¹⁵ In the large prospective Physicians' Health Study, 7% of cases of myocardial infarction (MI) were attributable to increased homocysteine levels.¹⁶ In another study, 28% of patients with CAD had homocysteine levels above the 90th percentile for controls. Of interest, familial aggregation was observed in one half of these cases.¹⁷ The association between increased homocysteine levels and atherosclerotic vascular disease is

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Table 1. Novel Risk Factors for Atherosclerosis

Homocysteine
Fibrinogen
Impaired fibrinolysis
Platelet reactivity
Hypercoagulability
Lipoprotein(a)
Small dense low-density lipoprotein
Infectious agents
Markers of inflammation
C-reactive protein
Soluble intercellular adhesion molecule 1

independent of traditional risk factors. However, homocysteine may have important interactions with other risk factors. Hyperhomocysteinemia substantially increases the risk associated with smoking and hypertension.^{13,18,19}

The relationship between homocysteine and risk of CAD seems to be linear, rather than having a threshold effect. When logistic regression is applied, the increased odds ratios for CAD are seen at total homocysteine levels within the range currently considered normal.²⁰ Severity of atherosclerotic disease in young patients with peripheral arterial disease is associated with high levels of homocysteine after a loading dose of methionine and high fasting homocysteine concentrations.¹⁹ Additionally, increased homocysteine levels seem to predict mortality in patients with CAD. In a prospective study involving 587 patients with CAD who were monitored for 4 years, cardiovascular mortality was 4.5 times higher for those with homocysteine levels greater than 20 µmol/L than for those with the lowest levels.²¹ Estimated survival in this study was related to homocysteine levels in a direct graded manner.

Mechanisms of Increased Risk

In vitro studies suggest several potential mechanisms of vascular damage mediated by high homocysteine levels: endothelial cell damage,²² mitogenic effect on vascular smooth muscle cells,^{23,24} activation of factor V,²⁵ inhibition of protein C,²⁶ tissue-type plasminogen activator (tPA) activation,²⁷ and enhanced platelet aggregation.²⁸ In humans, hyperhomocysteinemia is associated with endothelial dysfunction.²⁹⁻³¹ Because endothelial dysfunction may be an early step in the pathogenesis of atherosclerosis, these studies highlight an important mechanism by which hyperhomocysteinemia is atherogenic.

Clinical Implications

Screening for hyperhomocysteinemia should be considered in certain situations (Table 2). Prospective trial data that demonstrate a reduction in vascular events as a result of treatment of hyperhomocysteinemia are not yet available. In the European Concerted Action on Thrombosis

and Disabilities (ECAT)¹³ study and the Nurses' Health Study,³² participants who took vitamin supplements containing folate and the B vitamins had fewer risks of cardiovascular events. However, prospective randomized trials are needed to prove a beneficial effect of increased folic acid intake in the setting of secondary prevention.

The optimal method of decreasing homocysteine levels in patients at risk for CAD has not yet been determined.⁶ An algorithm for the treatment of hyperhomocysteinemia is shown in Figure 1. Of note, in patients with renal failure, much higher doses (up to 20 mg) of folate are needed to lower homocysteine levels.³³ Since January 1998, cereal grain or flour has been fortified with 140 µg of folate per 100 g of flour to reduce the incidence of neural tube defects. However, such supplementation may not adequately reduce risk of CAD.³⁴

FIBRINOGEN

Several studies point to the importance of fibrinogen as a risk factor for atherosclerotic vascular disease. A meta-analysis of 6 prospective epidemiological studies confirmed the association of increased fibrinogen levels with subsequent MI or stroke.³⁵ Persons with levels in the upper tertile had a relative risk of future cardiovascular disease that was 2.3 times higher than the risk in persons with levels in the lowest tertile. Of these 6 studies, only the Framingham Study included women.^{36,37} For men, the fibrinogen risk ratio was greatest for stroke, intermediate for MI, and smallest for peripheral arterial disease, whereas in women, the risk ratio was greatest for CAD. Fibrinogen levels were associated with conventional CAD risk factors. When these factors were included in the multivariate analysis, the association between fibrinogen and cardiovascular disease was weakened but remained statistically significant. In the Göttingen Risk Incidence and Prevalence Study, multivariate logistic regression analysis indicated the following as predictors of CAD: low-density lipoprotein cholesterol (LDL-C), family history, Lp(a), high-density lipoprotein cholesterol (HDL-C), fibrinogen, age, smoking, glucose, and blood pressure.³⁸ In patients with chronic angina, fibrinogen levels predicted subsequent acute coronary events.³⁹ Patients with low fibrinogen levels have a low risk of coronary events despite increased serum cholesterol levels.

Plasma levels of fibrinogen are, in part, genetically determined.^{40,41} Men tend to have higher levels than women, and black persons tend to have higher levels than white persons.⁴² Several environmental factors also affect the levels; smoking is the most important. The effect is dose related⁴³ and reversible on smoking cessation.⁴⁴ In the Framingham Study, almost 50% of the cardiovascular risk attributable to smoking was mediated through an increase

in plasma fibrinogen levels.⁴⁵ Fibrinogen levels increase with age, body size, diabetes, LDL-C, Lp(a), leukocyte count, and menopause.³⁵

Mechanism of Increased Risk

Fibrinogen may mediate its proatherogenic effects by increasing plasma viscosity, promoting platelet aggregability, and stimulating smooth muscle proliferation.⁴⁶ The role of fibrinogen as an acute phase reactant also must be considered as more evidence accumulates to suggest that atherosclerosis is an inflammatory process. In the ECAT study, high concentrations of fibrinogen and C-reactive protein (CRP) were associated with an increased coronary risk, suggesting that fibrinogen levels are elevated, at least in part, as a consequence of inflammatory changes that occur with progressive atherosclerosis.³⁹ Once increased, fibrinogen may aggravate underlying vessel wall injury and, by its procoagulant actions, predispose to further coronary events.

Clinical Implications

Fibrinogen screening may be helpful in creating risk profiles of patients who have atherosclerotic vascular disease in the absence of conventional risk factors. However, no data have yet suggested that reduction of fibrinogen levels would lead to altered clinical outcome. Fibrinogen levels are reduced by smoking cessation, exercise, alcohol intake, and estrogens.^{42,47} No drug lowers fibrinogen levels specifically. The fibrates perhaps have the most potent fibrinogen-lowering potential,⁴⁸ but these drugs are not suitable for examining the effect of fibrinogen reduction because they concomitantly reduce blood lipids.

IMPAIRED FIBRINOLYSIS

The fibrinolytic system consists of plasminogen, which is converted to its active form plasmin by plasminogen activators, including tPA. Inhibitors of the system include plasminogen activator inhibitor type 1 (PAI-1) and plasmin inhibitors such as α_2 -antiplasmin. In the Northwick Park Heart Study, fibrinolytic activity was measured by dilute blood clot lysis time at study entry in 1382 men, age 40 to 64 years, of whom 179 subsequently experienced episodes of CAD during a mean follow-up of 16.1 years.⁴⁹ In the men who were 40 to 54 years old, impaired fibrinolysis was associated with a significantly increased risk of CAD ($P=.002$), even after adjustment for plasma fibrinogen. No significant association was noted in older men.⁵⁰

In the Physicians' Health Study, higher concentrations of tPA were present in a nested case-control analysis of 231 participants who experienced MI compared with controls.⁵¹ In a multivariate analysis that controlled for conventional atherosclerotic risk factors, the association between tPA

Table 2. Indications for Measuring Plasma Homocysteine

Early-onset atherosclerosis (women <65 y, men <55 y)
Atherosclerosis in the absence of conventional risk factors
Thrombophilia
Chronic renal failure
Organ transplantation
Use of medications that may increase plasma homocysteine (eg, methotrexate)
Disorders with increased cell turnover (eg, psoriasis and malignant neoplasms)

antigen and risk of MI was no longer statistically significant, suggesting that the increase was a consequence of, rather than the cause of, atherosclerotic CAD. A separate analysis of the same group of subjects revealed that men with tPA antigen concentrations above the 95th percentile of control distributions had a risk of stroke that was almost 4 times that in men with lower concentrations, and the relationship remained significant after controlling for the accepted stroke risk factors and hyperlipidemia.⁵² Thus, baseline concentrations of tPA antigen may identify persons at high risk of stroke in whom aggressive risk factor modification may be indicated.

In the ECAT trial, a prospective study of more than 3000 patients with angina who were followed up for 2 years, tPA antigen levels at study entry were associated with a stepwise increase in risks of future MI and sudden death.⁵³ The risk associated with tPA was greater in magnitude than for any other hemostatic or thrombotic factor, including PAI-1 and factor VIII levels. Plasminogen activator inhibitor type 1 activity was prognostic in some stud-

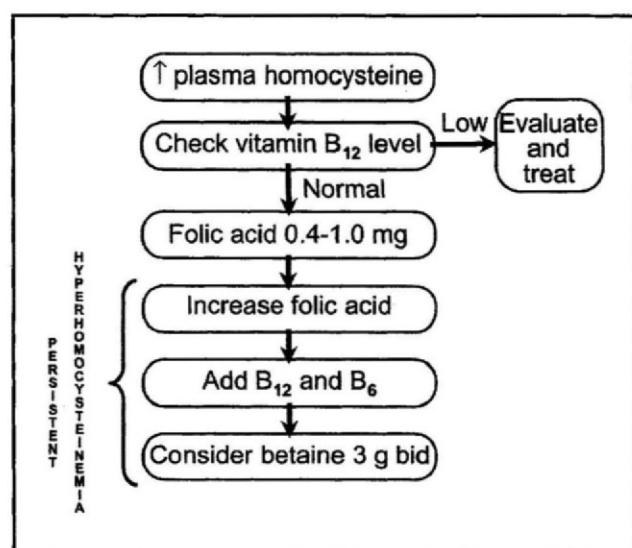


Figure 1. Algorithm for the treatment of hyperhomocysteinemia. bid = twice daily.

ies,^{54,55} but not in others.⁵⁶⁻⁵⁸ In the ECAT study, an increased incidence of events was associated with higher baseline concentrations of PAI-1 antigen ($P=.02$) and PAI-1 activity ($P=.001$).

Mechanism of Increased Risk

Increased tPA levels indicate an activated fibrinolytic system and therefore are a marker for ongoing thrombosis. In contrast, impaired fibrinolysis may contribute to atherosclerosis by enhanced fibrin deposition.⁵⁹ Insulin seems to regulate PAI-1 levels, and impaired fibrinolytic activity is a feature of insulin resistance.⁵³ Hypertriglyceridemia is also associated with increased PAI-1 levels.^{54,60}

Clinical Implications

Although the aforementioned studies provide insight into the possible contribution of impaired fibrinolysis in the pathogenesis of atherosclerotic vascular disease, the clinical utility of measuring factors of fibrinolysis is unknown. If markers for impaired fibrinolysis are identified in young persons at otherwise high risk, pharmacological and non-pharmacological methods may be used to enhance the fibrinolytic profile and possibly lessen the risk of CAD developing. Lifestyle changes may help to improve the fibrinolytic profile; fibrinolytic activity is inversely associated with smoking and obesity and positively associated with exercise.⁶¹ Pharmacological modulation of the fibrinolytic system is also possible. The renin-angiotensin system seems to have an important role in the regulation of vascular fibrinolysis,⁶² and angiotensin-converting enzyme inhibitors may enhance plasma fibrinolytic variables.⁶³ An increase in tPA levels and fibrinolytic potential may be an important mechanism whereby moderate alcohol consumption decreases the risk of heart disease.⁶⁴ Gemfibrozil lowers synthesis of PAI-1 and may enhance endogenous fibrinolysis.⁶⁵ Improved fibrinolytic potential may result from estrogen replacement therapy in postmenopausal women.⁶⁶

PLATELET REACTIVITY

Platelet size, function, and number may be related to the risk of developing atherosclerotic cardiovascular events. Cross-sectional data from the Caerphilly Collaborative Heart Disease Study, in which platelet aggregation was measured in about 2000 men with no history of recent aspirin ingestion, demonstrated that patients with CAD had a significantly greater adenosine diphosphate-induced platelet aggregation compared with controls.⁶⁷ Spontaneous platelet aggregation was a useful marker for survival and secondary coronary events among a cohort of patients followed up for 5 years after their index MI.⁶⁸ In a prospective study of 487 apparently healthy Norwegian

men followed up for a decade for the occurrence of vascular disease, a statistically significant rate of cardiovascular mortality was observed among subjects with the highest platelet counts (relative risk, 2.5 for those in the top quartile; $P=.002$). The association remained significant after adjustment for concurrent cigarette smoking in a multivariate analysis.⁶⁹

Mechanism of Increased Risk

Platelets have an important role in atherosclerosis.⁷⁰ Furthermore, the link between platelet function and CAD is underscored by the efficacy of aspirin in both primary and secondary prevention in at least 25 trials.⁷¹

Clinical Implications

Although diverse measurements of platelet function exist, these are technically difficult to perform. Physicians must distinguish between spontaneous platelet aggregation, which is induced by circulating agonists in the blood, and the response of platelets to agonists added externally. Flow cytometry to measure P-selectin, a marker of platelet activation, seems to be a promising indicator of increased platelet aggregation *in vivo* and needs to be assessed for its predictive value in prospective studies.⁷² Once increased platelet activation has been confirmed, persons at risk could take aspirin and newer antiplatelet agents and undergo other pharmacological, dietary, and behavioral interventions. Platelet aggregation in response to various agonists may be lessened by improved glycemic control in diabetic patients,⁷³ smoking cessation,⁷⁴ diets with high ratios of polyunsaturated to saturated fats,⁷⁵ and increased intake of ω -3 fatty acids,⁷⁶ alcohol,^{77,78} and vitamin E.⁷⁹

HYPEROAGULABILITY

An interesting feature of the hypercoagulable states is that they affect various vascular beds differently.⁸⁰ Despite the predisposition to venous thrombosis, congenital deficiencies of antithrombin III, protein C, and protein S are not associated with an increased incidence of arterial thrombosis or CAD.⁸¹ The factor V Leiden mutation is associated with venous thrombosis but not with atherosclerotic arterial disease, except perhaps in young women who smoke.^{82,83} The prothrombin gene mutation G20210A predisposes to venous thrombosis⁸⁴ but may also be a risk factor for MI.⁸⁵ Antiphospholipid antibodies have been implicated in both arterial thrombosis and venous thrombosis.⁸⁶ An association with CAD has also been described.⁸⁷

Mechanism of Risk

Clearly a hypercoagulable state would predispose to a greater thrombus burden in the presence of plaque rupture. This could lead to acute vascular syndromes and facilitate

progression of atherosclerosis.⁸⁸ However, the consequences of a hypercoagulable state seem to vary in different vascular beds, probably because of the differences in vascular phenotype and in the other components of the hemostatic-fibrinolytic system.⁸⁹

Clinical Implications

A clear-cut association between CAD and the hypercoagulable states has not yet been defined. In patients who have atherosclerotic vascular disease in the absence of conventional risk factors, a work-up for a hypercoagulable state may be worthwhile, although it is still unclear whether treatment with an anticoagulant such as warfarin would reduce the risk.

LIPOPROTEIN(a)

Lipoprotein(a) refers to a family of lipoprotein particles similar to LDL in core lipid composition and in having apo B-100 as a surface apolipoprotein. In addition, Lp(a) possesses a unique glycoprotein, apo(a), which is bound to apo B-100.⁹⁰ Lipoprotein(a) is associated with an increased risk of premature cardiovascular disease, but its physiological role remains obscure. Plasma levels vary widely among persons (about 1000-fold) but are stable within individuals, suggesting a strong heritable component.⁹⁰ Levels are generally unrelated to those of other lipoproteins and apolipoprotein.⁹⁰ The precise mechanisms that regulate Lp(a) levels are unknown. Lipoprotein(a) levels are increased in renal insufficiency, in the nephrotic syndrome, after kidney transplantation, and in patients undergoing hemodialysis or peritoneal dialysis. Fluctuations in Lp(a) seem to occur in states of hormonal change, such as in diabetes mellitus, after estrogen treatment, and during pregnancy. Black persons have higher levels of Lp(a) compared with white persons.⁹¹

Increased plasma Lp(a) is an independent risk factor for the development of premature coronary heart disease in men, comparable in magnitude (ie, attributable risk) to a total cholesterol level of 240 mg/dL or more or to an HDL level less than 35 mg/dL.⁹² Plasma concentrations greater than 20 mg/dL have been reported to increase the risk of CAD, cardiovascular accident, and peripheral arterial disease. Plasma Lp(a) levels correlate with the number and severity of coronary lesions in men undergoing coronary arteriography for clinically suspected coronary atherosclerosis.⁹³

A series of cross-sectional and retrospective case-control studies support a role for Lp(a) as a risk factor for CAD. Most retrospective studies show that levels of plasma Lp(a) are higher in patients with CAD than in controls. An Lp(a) level greater than 39 mg/dL was the most common form of familial dyslipidemia in a study of

patients with confirmed CAD before age 60 years.⁹⁴ Several prospective studies have analyzed the relationship between baseline Lp(a) levels and future vascular events. In a prospective study of 4849 men followed up for 8 years (Prospective Cardiovascular Munster Study), multiple logistic function analysis revealed that, in addition to conventional risk factors, Lp(a) predicted an increased risk of major coronary events.⁹⁵ In the Göttingen Risk Incidence and Prevalence Study, which monitored 6002 men ages 40 to 60 years for 5 years, multivariate logistic regression models confirmed Lp(a) as an important risk factor, ranking fifth behind LDL-C, family history of MI, plasma fibrinogen, and low HDL-C.⁹⁶ A nested case-control study from a cohort of participants in the Lipid Research Clinics Coronary Primary Prevention Trial⁹⁷ showed that the Lp(a) level is an independent risk factor for CAD in white men with hypercholesterolemia. However, in the Physicians' Health Study, Lp(a) levels failed to predict cardiovascular events.⁹⁸ The conflicting data from the prospective studies may be the result of variability in the methods used to determine Lp(a) levels and the fact that different isoforms of the apo(a) component of Lp(a) might have different atherogenic potential.

The association of Lp(a) with risk of CAD is also present in women. In a cohort of 9936 men and women initially free of cardiovascular disease who were followed up for 14 years, Lp(a) was a significant predictor of risk of future CAD in both sexes.⁹⁹ In another study, Lp(a) was a determinant of CAD in both premenopausal and postmenopausal women.¹⁰⁰ The increased risk of CAD in postmenopausal women may be due, in part, to a 25% increase in Lp(a) levels that occurs after menopause.¹⁰¹ Although black persons have a higher median plasma Lp(a) concentration than white persons, this does not seem to translate into an increased risk of CAD.⁹¹ The risk of CAD may be increased synergistically in patients with increased Lp(a) levels and low levels of HDL-C.¹⁰² No such interaction, however, was observed in the Framingham Offspring Study.⁹²

Mechanism of Increased Risk

Stable levels of Lp(a) are attained during infancy and are maintained throughout life. This may explain the strong correlation of increased Lp(a) levels with premature CAD. Atherogenic effects of Lp(a) may result from the LDL-like properties and delivery of cholesterol at sites of vessel injury.¹⁰³ As in the case of LDL, oxidation of Lp(a) seems to confer increased atherogenicity.¹⁰⁴ Lipoprotein(a) also has thrombogenic properties from the apo(a) moiety, which is structurally similar to plasminogen but lacks fibrinolytic activity. By competing with plasminogen in binding to fibrin, Lp(a) may hinder endogenous fibrinolysis.^{105,106} Such an impairment in fibrinolysis with increased Lp(a)

levels has been implicated in rapid angiographic progression of CAD and decreased coronary patency after thrombolysis in patients with MI¹⁰⁷ and for restenosis after percutaneous balloon coronary angioplasty.¹⁰⁸ Lipoprotein(a) has been shown to stimulate the growth of human vascular smooth muscle cells in vitro,¹⁰⁹ enhance expression of intercellular adhesion molecule 1 (ICAM-1) in endothelial cells,¹¹⁰ inhibit activation of plasminogen by tPA, and increase expression of PAI-1.

Clinical Implications

Lipoprotein(a) levels should be determined in patients who have premature CAD or a family history of premature CAD or in those who develop CAD in the absence of conventional risk factors.⁹⁰ Lipoprotein(a) exerts its pathologic effect at plasma levels greater than 20 to 30 mg/dL.¹¹¹ Levels may need to be measured only once because variability is small within an individual person.⁹⁰ If increased levels are detected, testing and counseling of family members may be warranted. In considering these recommendations, the physician must be aware that current assays are limited because of an inability to account for the genetic heterogeneity of Lp(a), which may result in production of isoforms of varying molecular weights with differing atherogenic potential.

Pharmacological therapy for increased Lp(a) levels has been only modestly successful; apheresis has been the most effective therapeutic modality.¹⁰³ 3-Hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitors do not lower Lp(a) levels.¹¹² However, lowering of LDL levels may decrease the pathogenicity of increased Lp(a) levels. Maher et al¹¹³ found that, in patients with combined increases of LDL-C and Lp(a), a substantial reduction in angiographic progression of CAD and in clinical event rates occurred with a substantial lowering of LDL with no change in Lp(a) levels. Estrogens decrease plasma Lp(a) concentrations, which could explain some of the vascular protective effects of estrogen replacement therapy.¹¹⁴ Niacin,¹¹⁵ gemfibrozil,¹¹⁶ and ω-3 fatty acids¹¹⁷ lower Lp(a) levels to various degrees but not consistently. Detection of high Lp(a) levels in a patient with CAD or at risk of developing CAD may be an indication for more aggressive therapy, particularly in an attempt to lower LDL-C levels.¹¹⁸

SMALL DENSE LDL

Low-density lipoprotein particles differ in size and density and have been divided into 2 distinct phenotypes: pattern B with a predominance of small dense LDL particles and pattern A with a higher proportion of large, more buoyant LDL particles.¹¹⁹ Small dense LDL particles tend to coexist with increased triglycerides and low HDL-C values, a combination that has been termed *atherogenic dyslipidemia*.¹¹⁹

Several retrospective studies have suggested that pattern B is associated with an increased risk of CAD¹²⁰⁻¹²³; however, they did not clearly establish whether this effect is independent of the coexisting lipoprotein abnormalities. Three recent prospective studies have attempted to resolve this issue. A nested case-control study from the Stanford Five-City Project showed that LDL particle size is associated inversely with CAD, even after adjustment for other risk factors including triglycerides and HDL-C.¹²⁴ In the Physicians' Health Study, small dense LDL was associated with an increased risk of MI but not after adjustment for the triglyceride level.¹²⁵ In the Quebec Cardiovascular Study, LDL phenotypes were analyzed in 114 cases of CAD and matched controls from a cohort of 2103 men followed up for 5 years.¹²⁶ Conditional logistic regression revealed that men whose LDL particle diameter was in the first tertile had a 3.6-fold increase in the risk of CAD compared with those whose LDL particle diameter was in the third tertile. The relationship remained significant after adjustment for variations in LDL-C, triglycerides, HDL-C, and apo B concentrations. Thus, the preponderance of evidence indicates an independent association of small LDL size to CAD.

Mechanism of Increased Risk

Animal and in vitro studies demonstrate that small dense LDL has increased atherogenicity. This may be due to its increased ability to cross into the subintimal space, greater susceptibility to oxidation,¹²⁷ and increased binding to intimal proteoglycans.¹²⁸

Clinical Implications

Low-density lipoprotein density is currently measured only in specialized laboratories. Triglyceride levels may serve as a surrogate marker for LDL density, with high levels indicating the presence of the small dense phenotype. In subjects with normal or slightly elevated triglyceride levels, determination of the LDL phenotype may be helpful in risk stratification. Additionally, the small dense LDL phenotype may help to predict response to CAD risk-reduction approaches. In the Stanford Coronary Risk Intervention Project, an angiographic trial that compared multifactorial risk-reduction intervention with usual care, the beneficial effect on disease progression after 4 years was observed almost entirely in men with the dense LDL phenotype.¹²⁹ Bile acid resins were the drugs most frequently used for lipid lowering in this study, although lipid changes did not account for this difference in angiographic response. In contrast, in the Monitored Atherosclerosis Regression Study, lovastatin resulted in angiographic benefit only in men with the larger buoyant LDL particles.^{130,131} Profiling LDL particle phenotype may help to predict re-

response to various lipid-lowering agents and help in tailoring optimal therapy for individual patients.

Modification of LDL size may involve different approaches than those typically taken to lower LDL-C.¹³² Favorable increases in LDL particle diameter have been seen after an intensive exercise-training program.¹³³ Simvastatin has no effect on LDL particle size,¹³⁴ but ciprofibrate¹³⁵ and niacin¹³² may favorably alter LDL particle size.

MARKERS OF INFLAMMATION

C-Reactive Protein

C-reactive protein, an acute phase reactant, is a marker for underlying systemic inflammation. Several studies have established an association between CRP and various manifestations of atherosclerosis. In a cross-sectional study, levels of CRP were associated with several other vascular risk factors, including smoking, hypertension, hypercholesterolemia, obesity, and diabetes.¹³⁶ Levels of CRP were increased in chronic angina and in acute coronary syndromes.¹³⁷ Increased CRP levels may predict vascular events, as highlighted by recent prospective studies. In the Multiple Risk Factor Intervention Trial,¹³⁸ increased CRP levels were associated with increased risks of CAD, but this relationship was detected only in smokers. In the Physicians' Health Study, a prospective study of apparently healthy male physicians, baseline levels of CRP predicted future risk of MI and thromboembolic stroke.¹³⁹ The men in the quartile with the highest CRP values had 3 times the risk of MI and 2 times the risk of ischemic stroke compared with men in the lowest quartile. Levels of CRP were increased many years before occurrence of vascular events. The risk of future events associated with CRP was independent of conventional risk factors as well as Lp(a), fibrinogen, tPA antigen, and homocysteine. Baseline levels of CRP also predicted the development of peripheral vascular disease.¹⁴⁰

The CRP may be an even stronger predictor of vascular events in women. In a nested case-control study from the Women's Health Study,¹⁴¹ 122 patients who had MI or cerebrovascular accident were compared with 244 controls matched for age and smoking status. The adjusted relative risk of MI or stroke for women with CRP levels in the highest quartile was 5.5 compared with 2.8 for men participating in the Physicians' Health Study.

Plasma CRP levels are associated with the extent and severity of atherosclerotic vascular disease.^{142,143} In patients with known CAD, increased levels of CRP are associated with an increased risk of future coronary events.¹⁴⁴ These associations remained significant even after adjustment for body mass index, smoking history, hypertension, and total cholesterol.

Mechanism of Increased Risk.—The pathophysiological role of CRP in atherosclerosis has not been defined. The presence of CRP immunoreactivity has been demonstrated in vulnerable and ruptured coronary artery plaques¹⁴⁵; whether this is expressed locally or absorbed from the bloodstream is unclear. C-reactive protein may have prothrombotic effects by increasing expression of tissue factor.¹⁴⁶ C-reactive protein can activate complement,¹⁴⁷ and it is directly related to interleukin 6 levels, a cytokine that promotes leukocyte adhesion to the vasculature.

Clinical Implications.—In the Physicians' Health Study, predictive models incorporating both lipid and CRP levels provided a better risk assessment than did models in which only lipids were used.¹⁴⁸ Furthermore, CRP levels predicted vascular events in patients with normal lipids. Because an association between the use of aspirin and reduction in the risk of a first MI was directly related to the levels of CRP, measurement of levels may have clinical utility in determining patients who are likely to benefit from aspirin and perhaps newer anti-inflammatory agents.¹³⁹

Soluble Intercellular Adhesion Molecule 1

Soluble ICAM-1 was associated with an increased risk of MI in the Physicians' Health Study.¹⁴⁸ The risk of future MI was 80% higher in men with the highest baseline quartile of ICAM-1 compared with men in the lowest quartile. Soluble ICAM-1 levels were associated with fibrinogen, HDL, homocysteine, triglycerides, tPA antigen, and CRP. The association between CAD and soluble ICAM-1 remained significant even after adjustment of these factors as well as smoking and high cholesterol levels. The source of increased levels of ICAM-1 is unclear, but it is likely to be vascular wall cells.

Mechanism of Risk.—Intercellular adhesion molecule 1 mediates adhesion and transmigration of monocytes to the vessel wall.¹⁴⁹ Increased levels likely indicate endothelial cell activation and inflammation. The strongest plasma correlate of soluble ICAM-1 in the Physicians' Health Study was CRP, another marker of inflammation. Although the biologic role of CRP is uncertain, soluble ICAM-1 may be directly involved in various stages of atherogenesis.

Clinical Implications.—Increased levels of soluble ICAM-1 and other cell adhesion molecules may serve as markers of early atherosclerosis and thereby of increased risk of CAD. Additionally, therapies to block the effects of adhesion molecules may be a novel approach to the inhibition of atherogenesis.

INFECTIOUS AGENTS

Recently, increasing interest has centered on the infectious theory of atherosclerosis. The hypothesis was initially pro-

Table 3. Evidence Supporting Involvement of Infectious Agents in Atherosclerosis*

Evidence	Presence or absence of evidence for an infectious agent		
	Cytomegalovirus	<i>Herpesvirus hominis</i>	<i>Chlamydia pneumoniae</i>
Seroepidemiology			
Atherosclerosis	+	-	+
Transplantation arteriosclerosis	+	-	-
Pathogen present in atheroma	+	±	+
Can produce atheroma in animals	+	+	±
Proof of causality	-	-	-

*Modified from Libby et al¹⁵⁵ with permission.

posed in the first 2 decades of the 20th century,^{150,151} but it was not until the study by Fabricant et al¹⁵² in 1978, demonstrating development of atherosclerosis in chickens infected with the avian herpesvirus, that interest was rekindled. Subsequently, *Chlamydia pneumoniae*, *Helicobacter pylori*, *Herpesvirus hominis*, and cytomegalovirus have been implicated as primary etiologic factors or cofactors in the pathogenesis of atherosclerosis.^{153,154} Evidence thus far has consisted of demonstrating the presence of the agent in atherosclerotic lesions by immunocytochemistry or molecular biology or associating atherosclerotic disease and positive serologic test results for an infectious agent¹⁵³ (Table 3). A recent study demonstrated that intranasal *C pneumoniae* infection accelerated atherosclerosis in cholesterol-fed rabbits. Additionally, treatment with azithromycin after infectious exposure prevented the accelerated atherosclerosis.¹⁵⁶

Mechanism of Increased Risk

The pathophysiological mechanisms by which infectious agents could lead to atherosclerosis were reviewed by Libby et al.¹⁵⁵ These mechanisms include production of proinflammatory mediators such as cytokines and free radical species, stimulation of smooth muscle cell proliferation, mononuclear cell and T-lymphocyte proliferation, and endothelial dysfunction with associated procoagulant and proadhesive phenotype. Additionally, activation of an infectious organism within a chronic lesion might lead to plaque inflammation and destabilization and acute syndromes.¹⁵⁷

Clinical Implications

Infectious agents may be etiologic factors or cofactors in the pathogenesis of atherosclerosis or perhaps simply bystanders. The infectious etiology of atherosclerosis remains an intriguing hypothesis. Further research is needed to substantiate and confirm the hypothesis. Recently, Gupta et al¹⁵⁸ demonstrated that an increased anti-*C pneumoniae* antibody titer may be a predictor for further

adverse cardiovascular events in patients who have had an MI and that a brief course of azithromycin may lower this risk, possibly by acting against *C pneumoniae*. In ROXIS (randomized trial of roxithromycin in non-Q-wave coronary syndromes), a double-blind placebo-controlled pilot study, 202 patients with unstable angina or non-Q-wave MI were randomized to receive roxithromycin or placebo for 30 days.¹⁵⁹ At 1 month, the composite end point of ischemic death, MI, and severe recurrent ischemia was significantly less in the antibiotic treatment group. The investigators postulated that roxithromycin may stabilize plaque by its antichlamydial activity or through its anti-inflammatory properties. These are interesting but only preliminary findings that must be validated by large-scale trials.

CONCLUSION

Atherosclerotic vascular disease is an exceedingly common disorder whose etiology is complex and multifactorial. Identifying new risk factors for atherosclerosis is important to enhance our understanding of its etiology, improve our ability to predict risk, and facilitate development of therapeutic and preventive approaches. Although pharmacological treatment is possible for several of the novel risk factors discussed in this article, prospective trial evidence to support such an approach is awaited. In the meantime, identification of novel risk factors may improve risk stratification and motivate the patient and physician to adopt an aggressive approach to the treatment of conventional risk factors.

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