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Assessment of Hemostatic Risk Factors in Predicting Arterial Thrombotic Events

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Abstract—Arterial thrombosis results from endovascular injury and, to a lesser extent, alterations in hemostatic equilibrium. Although multiple hereditary and acquired hemostatic risk factors have been described in the pathophysiology of venous thrombosis, the degree and type of abnormalities that contribute to arterial thrombosis are less well understood. Endothelial cell injury with the elaboration of proinflammatory mediators stimulates the process of arterial thrombosis. Although this is most often the result of endovascular injury attributable to atherosclerotic disease, other disease states can elicit a similar response as well. Similarly, once thrombosis has been initiated, variations in the activity of coagulation proteins and endogenous anticoagulants, as well as the kinetics of platelet aggregation, may alter the effectiveness of thrombus formation. Epidemiological studies have identified several acquired or inherited states that may result in endothelial damage or altered hemostatic equilibrium, thereby predisposing patients to arterial thrombosis. These include hyperhomocysteinemia, elevated C-reactive protein, antiphospholipid antibodies, elevated fibrinogen, Factor VII, plasminogen activator inhibitor-1 (PAI-1), hereditary thrombophilias, and platelet hyper-reactivity. This review explores our present understanding of these risk factors in the development of arterial thrombotic events. At present, the literature supports a role for hyperhomocysteinemia, elevated C-reactive protein, and elevated fibrinogen as risk factors for arterial thrombosis. Similarly, the literature suggests that lupus anticoagulants and, to a lesser extent, elevated titers of cardiolipin IgG antibodies predispose to arterial vascular events. In certain subsets of patients, including those with concomitant cardiac risk factors, $<$55 years of age, and women, hereditary thrombophilias such as carriership of the factor V Leiden and the prothrombin G20210A mutations may confer a higher risk of arterial thrombosis. However, the data on Factor VII, PAI-1, and platelet receptor polymorphisms are contradictory or lacking.
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Key Words: arterial ■ thrombosis ■ atherosclerosis ■ hemostasis ■ risk factors

Hereditary and acquired hemostatic risk factors continue to be the focus of active research and interest as investigators seek to understand their role in the development of vascular pathology, morbidity, and mortality. In recent years, an increased understanding of genetics and molecular biology has fueled this research and shed light on several

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hemostatic risk factors that may help to predict arterial thrombotic events. This review explores our present understanding of the role of hyperhomocysteinemia, elevated C-reactive protein (CRP), antiphospholipid antibodies (APLA), elevated fibrinogen, Factor VII, plasminogen activator inhibitor-1 (PAI-1), hereditary thrombophilias, and platelet hyper-reactivity in predicting the development of arterial thrombosis.

Homocysteine

Homocysteine is a reactive amino acid intermediate in methionine metabolism. Once formed, homocysteine can either be remethylated by methionine synthetase, a vitamin B_{12} and folate-dependent enzyme, to form methionine, or, alternatively, it can be transulfurated by cystathionine–β-synthase, a B_6-dependent enzyme, to form cysteine. The potential role of plasma total homocysteine (tHcy) in the development of atherosclerotic vascular disease was first recognized in 1969 by McCully,[1] who described fibrous vascular plaques in 2 children, each with different enzymatic defects that resulted in severe hyperhomocysteinemia. In 1975, Wilcken and Wilcken[2] demonstrated impaired homocysteine metabolism in a cohort of patients with premature coronary artery disease (CAD). In the intervening year, many studies have found a modest but consistent association between tHcy and arterial vascular outcomes.

More recently, Wald et al[3] performed a meta-analysis of 72 prospective cohort studies that examined the association between carriership of the methylenetetrahydrofolate reductase (MTHFR) C677T gene, the most common form of genetic hyperhomocysteinemia, tHcy, CAD, stroke, and venous thromboembolism. Of the 46 studies that examined the end point of CAD, individuals with the MTHFR 677 TT allele had a summary odds ratio (OR) of 1.21 (95% CI, 1.06 to 1.39) for CAD compared with the CC wild type, and there were no significant differences in serum cholesterol, body mass index (BMI), blood pressure, or smoking between these groups. Similar but less robust results were found by Klerk et al,[4] who reported an OR of 1.16 (95% CI, 1.05 to 1.28) for CAD among individuals with the TT genotype compared with individuals with the CC genotype. This mild association between tHcy and arterial thrombotic outcomes is consistent with similar studies on tHcy and coronary and cerebrovascular outcomes.[5–7]

It is now known that there is significant variability in serum homocysteine levels in the general population, that folate and vitamin B_{12} status is negatively correlated with fasting serum homocysteine concentration, and that serum homocysteine is positively correlated with increased age, serum creatinine, systolic blood pressure, male gender, cigarette smoking, and the consumption of coffee and possibly alcohol.[8,9] However, the mechanisms by which elevated levels of homocysteine lead to atherosclerotic vascular disease remain poorly understood. Hypotheses include impaired endothelium-dependent vasomotor regulation, increased endoplasmic reticulum stress, the formation of oxidized low-density lipoprotein (LDL), enhanced production of inflammatory mediators, stimulation of smooth muscle cell proliferation, and induction of a prothrombotic state through enhanced platelet activation, increased tissue factor expression, and inhibition of protein C.[10–13]

Despite reasonably convincing evidence of an association between serum homocysteine and vascular end points, to date there are no convincing data that demonstrate that therapies aimed at reducing serum homocysteine result in favorable outcomes. A recent secondary prevention trial in patients with stroke failed to demonstrate a benefit from vitamin therapy in reducing atherothrombotic outcomes,[14] but there are several other studies underway aimed at answering this question.[15,16] However, because of the recent adoption of mandatory folate supplementation in all grain-based products in the United States, there will be even less baseline variation among study participants and controls, which will make demonstrating statistically significant differences even more challenging.

C-Reactive Protein

CRP is an acute-phase reactant and a member of the pentraxin family: a highly conserved group of proteins characterized by their pore-forming cyclic structure and calcium-dependent ligand binding. CRP binds with high affinity to numerous endogenous and exogenous ligands, including phosphorylcholine, modified lipids, apoptotic cells, and microbial polysaccharides.[17,18] Although the majority of CRP is synthesized by hepatocytes in response to interleukin-6 (IL-6), it is also secreted by coronary artery smooth muscle cells in response to IL-1β, IL-6, tumor necrosis factor-α (TNF-α), and lipopolysaccharide.[19] Once bound, CRP is a potent stimulator of the classical complement pathway; it promotes macrophage phagocytosis as well as the production of transforming growth factor-β.[17] In recent years, CRP has emerged as a recognized cardiovascular risk factor because numerous studies have demonstrated that serum levels are positively correlated with cardiovascular outcomes, an association that is independent of traditional cardiac risk factors.[20,21]

There are several mechanisms by which CRP may contribute to the development of atherothrombotic vascular events. Elevated CRP levels cluster with traditional cardiovascular risk factors, including obesity, smoking, hypertension, type 2 diabetes, and exercise,[22–25] and there are data that suggest a direct effect of CRP on endothelial cells, including the upregulation of vascular adhesion molecules, stimulation of proinflammatory mediators, and the impairment of vascular NO-dependent vasodilation.[26]

Ridker et al,[27] in a prospective, nested case-control study of 1086 males participating in the Physicians Health Study,[28] demonstrated that CRP concentrations were predictive of future arterial vascular events. Men with elevated baseline plasma CRP levels (tertiles 0.56 to 1.14, 1.15 to 2.10, and $\geq$2.11 mg) had a relative risk (RR) of 1.7, 2.6, and 2.9, respectively, for future myocardial infarction (MI). The risk of future ischemic stroke followed a similar trend: 1.7, 1.9, and 1.9, and these associations persisted after adjustment for smoking, hyperlipidemia, diabetes, and hypertension. In a similar study, Ridker et al[29] found that among 27 939 women participating in the Women's Health Study (WHS),[30] baseline CRP levels were more predictive of the composite end points of coronary heart disease, stroke, and death from other cardiovascular causes than was serum LDL, a finding that

persisted after adjustment for traditional cardiac risk factors. Investigators have reported similar results in studies examining the association of CRP levels with the development of peripheral vascular disease[31] and recurrent atherothrombotic events, including ischemic stroke,[32] unstable angina, and MI.[33,34]

The epidemiological data support CRP as a strong independent risk factor for the development of atherothrombotic events; however, it remains unclear whether CRP is simply a marker of the systemic inflammation that is part of atherogenesis or is actually an instigator of vascular disease. Answering this question is difficult, but recent studies on the use of statins in patients with vascular disease provide further insight.

3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors or statins can reduce plasma levels of CRP by 13% to 50%.[35] Building on these observations, Ridker et al[36] performed a nested case-control study on the effects of statin therapy on CRP levels and the combined end points of recurrent MIs or death in a cohort of patients who had experienced acute MI. The patients who achieved a CRP level of <2 mg/L had 2.8 events per 100 patient years compared with patients who achieved CRP of >2 mg/L, who had 3.9 events per 100 patient years. The reduction in CRP levels was associated with a significant improvement in event-free survival that was additive and independent of the achieved LDL cholesterol level. These results are supported by Nissen et al,[37] who demonstrated a decreased rate of coronary artery plaque progression by reducing CRP levels.

CRP is an independent risk factor for arterial thrombotic events, and there are preliminary data that lowering CRP levels improves cardiovascular outcomes, but it is still not clear that the reduction in CRP levels completely explains the improvement in cardiovascular outcomes. In particular, statins are known to have pleiotropic effects, and it is possible that the lower CRP levels are a reflection of other immunomodulatory effects on the vascular endothelium.

Antiphospholipid Antibodies

APLA are a heterogeneous group of immunoglobulins directed against epitopes which result from the interaction of phospholipids and proteins such as annexin V, prothrombin, cardiolipin, and β_2-glycoprotein I (β_2-GPI).[38–40] Several of these autoantibodies are associated with an increased risk for the development of venous and arterial thromboembolism, but which subtypes predominate and the mechanisms by which these events occur are not fully understood. Of the known APLA, several are implicated in vascular events. Although there is often significant overlap between subtypes, several categories have been defined, including those directed against cardiolipin (anticardiolipin antibodies [aCL]), those causing prolongation of the partial thromboplastin time or lupus anticoagulants (LA), and those that bind β_2-GPI.

For a number of reasons, the exact role of aCL in the development of arterial thrombosis remains unclear. These include lack of uniformity in assays and interpretation, failure of previous studies to distinguish between venous and arterial thrombosis, and the preponderance of data from studies in patients with a primary diagnosis of connective tissue disease. In a systematic review, Galli et al[41] analyzed 5 prospective and 11 case-control and cross-sectional studies on aCL and the risk of thrombosis and found that only 50% of the studies that reported an association between aCL and thrombosis reached statistical significance. Two of the 5 prospective and 7 of the 11 case-control and cross-sectional studies demonstrated a statistically significant association between aCL and arterial events. In a separate analysis, it was found that elevated aCL IgG titers (>33 to 40 GPL units) were associated with an increased risk of arterial thrombosis, and that the higher the titer the higher the OR. However, the authors were clear that these data are limited by variability in study design, the aCL assays used, and the lack of uniform consensus on assay interpretation. Although it appears that mild to moderate elevations in aCL IgG are associated with arterial thrombosis, more rigorous research on this topic is indicated.

Despite its singular name, lupus anticoagulant (LA) actually comprises a subgroup of APLA that disrupt the in vitro assembly of the prothrombinase complex (factors Xa, Va, and prothrombin in complex on phospholipid membranes) and thus lead to prolongation of the activated partial thromboplastin time. Interestingly, this in vitro anticoagulant effect is associated with a paradoxical thrombotic diathesis in vivo. In the same review described above, Galli et al[41] report a summary OR of 5.7 to 9.4 (95% CI) for LA and the risk of venous and arterial thrombosis. However, these data are heavily weighted toward venous events, with only 1 study showing a risk between LA and ischemic stroke.

It has also been proposed that β_2-GPI antibodies are the primary LA subtype associated with thrombotic outcomes, but the data are inconclusive. In a recent study by Veres et al,[42] the authors report that the frequency and titers of anti–β_2-GPI antibodies were significantly higher in patients with acute coronary syndromes than in controls, data that are consistent with previous studies on patients with stable and unstable angina.[43] Building on these observations, de Laat et al[44] developed an assay to discriminate between β_2-GPI antibody–dependent LA and β_2-GPI–independent LA activity. They subsequently applied this assay to a retrospective cohort of 198 patients with either systemic lupus erythematosus (SLE; n=176), lupus-like disease (n=16), or primary antiphospholipid syndrome (n=6) and found that among the 58 patients with LA, 25 had β_2-GPI antibody–dependent LA activity. The OR of previous thromboembolic events within the β_2-GPI antibody–dependent LA group was 42.3 (95% CI, 9.9 to 194.3), whereas the OR for the 33 patients with β_2-GPI–antibody independent LA activity was 1.6 (95% CI, 0.8 to 3.9).[38] Although this study is intriguing, a recent review of 28 studies including 4394 patients and 1973 controls found that although there was an overall association between β_2-GPI antibodies and the risk of thrombosis, when analyzed with respect to the type of thrombosis (arterial versus venous), there was no statistically significant association between anti–β_2-GPI antibodies and arterial events.[45]

Because accelerated atherosclerosis is a well-recognized phenomenon in patients with SLE,[46,47] investigators have sought to understand the role of APLA in the pathogenesis of vascular injury. It has long been assumed that the development of vascular disease in patients with SLE is attributable to the systemic effects of the disease itself, as well as the

therapies aimed at halting its progression. For example, SLE patients with renal disease may develop hypertension and uremia and, when treated with immunosuppressive therapies such as corticosteroids, may develop insulin resistance and dyslipidemias, all of which are associated with arterial vascular disease. However, recent studies suggest a more complicated explanation for the increased risk of atherosclerosis and thrombosis seen among individuals with SLE.

In a retrospective cohort analysis of 2 SLE registries, Esdaile et al[48] report that after controlling for traditional Framingham risk factors (age, gender, blood pressure, diabetes, tobacco use), patients with SLE had an RR of 7.5 for CAD (95% CI, 5.1 to 10.4) and 7.9 for stroke (95% CI, 4.0 to 13.6) compared with controls. In addition, the authors found that the degree of atherosclerosis, as measured by carotid artery intimal plaque, was associated with a longer duration of disease, a higher damage-index score, and less aggressive immunosuppression. Similarly, Roman et al,[49] in a case-control study of 197 patients with SLE and 197 matched controls, found that after controlling for traditional cardiac risk factors, the prevalence of atherosclerosis was significantly higher among patients with SLE than in controls (37.1% versus 15.2%; $P<0.001$), and that these patients had more disease-related damage and were less likely to have been treated with immunosuppressant therapy.

These data would suggest that patients with more indolent disease, who are generally less likely to receive aggressive immunotherapy, may experience ongoing subclinical vascular injury, which may lead to premature atherosclerosis. Further supporting this hypothesis is the observation that patients who have circulating APLA, without underlying SLE or collagen vascular disease, are also at increased risk for atherosclerotic disease progression, morbidity, and mortality.[50–53] Whether these antibodies are a direct cause of atherosclerosis and subsequent thrombosis or an epiphenomenon attributable to the presence of immunogenic atherosclerotic plaques remains unclear.

Another area of interest has been the extent to which interactions between APLA and epitopes associated with oxidized LDL may contribute to atherogenesis.[54,55] The presence of β_2-GPI in LDL and oxidized LDL has led to the hypothesis that β_2-GPI antibodies may facilitate phagocytosis of oxidized lipid by foam cells in developing atheromas. The observation that some patients with SLE have elevated levels of antibodies against oxidized LDL has led others to postulate that there may be cross-reactivity between APLA directed against cardiolipin and β_2-GPI and oxidized LDL.[56] There are also small studies in patients with accelerated atherosclerosis that suggest that the presence of elevated titers of antibodies against oxidized LDL predict future cardiovascular events.[57] Although it remains plausible that these antibodies play a role in atherogenesis, the mechanisms remain unclear.

Although the literature supports a role for APLA in the development of arterial thrombotic events, a complete explanation has been challenging for several reasons. First, most studies are observational or retrospective cohort analyses and subject to multiple confounders. Second, APLA may occur as a distinct entity, as in the case of the primary antiphospholipid syndrome, or in the setting of an existing connective tissue disease, such as SLE. These distinctions are important because most of the literature on the role of APLA in arterial disease focuses on patients with APLA in the setting of connective tissue disease, where other disease processes may contribute to vascular events. Finally, there is wide historic variability in the designation of APLA subtypes, assays, and titers.

Fibrinogen

Fibrinogen is the only clotting factor for which there are compelling data supporting an association between plasma levels and the risk of vascular outcomes.[58,59] The fibrinogen genes α, β, and γ are located on chromosome 4 and contain promoter sequences that are responsive to IL-6 and hepatic nuclear factor-1, and suppressor sequences that are responsive to TNF-α and IL-1.[60,61] It is also known that fibrinogen levels are positively correlated with tobacco use.[62] The formation of the β-chain is the rate-limiting step in fibrinogen synthesis, and genetic polymorphisms in this gene have been identified that lead to elevated plasma levels of fibrinogen, the best described being the Bc/L and -455G/A polymorphisms located in the 3′ region of the β-chain.[61] Several studies have shown that the -455G/A polymorphism is associated with elevated plasma fibrinogen levels, especially in males and smokers.[63,64]

It is hypothesized that fibrinogen contributes to the development of atherosclerotic plaques by migrating into the intima of injured vessel walls, where it forms cross-linked fibrin, mural thrombi, and fibrin degradation products (FDP). This process leads to the elaboration of inflammatory mediators that promote cell migration and adhesion, platelet aggregation, smooth muscle cell proliferation, and foam cell lipid uptake.[59,65–67]

Lee et al,[68] in a case-control population survey of 10 359 men and women, found statistically significant elevated levels of fibrinogen among individuals with cardiac risk factors of family history, hypertension, and diabetes, as well as among individuals with angina or MI. Similarly, in the Framingham Offspring Study, investigators demonstrated a linear relationship between fibrinogen levels and traditional Framingham risk factors, including age, smoking, diabetes mellitus, total cholesterol, and high-density lipoprotein and LDL cholesterol.[69]

A number of prospective studies have examined the relationship between the fibrinogen levels and CAD outcomes. The Northwick Park Heart Study (NPHS) study followed 1511 men between the ages of 40 and 64 for 5 years, during which time there were 109 ischemic heart events. Analysis of the data demonstrated that for each SD increase in fibrinogen above the mean, there was an associated 84% increase in the 5-year risk of ischemic heart disease. Regression analysis found that this relationship remained even after controlling for confounders.[70] In the Prospective Cardiovascular Munster (PROCAM) study, 2116 healthy males were followed for 6 years, during which time 82 coronary events (9 sudden cardiac deaths and 14 fatal and 59 nonfatal MIs) were observed. The incidence of coronary events in the upper tertile of the plasma fibrinogen distribution was 2.4-fold higher than in the lower tertile. Multiple logistic regression

analyses demonstrated that plasma fibrinogen was an independent risk factor for CAD. Moreover, among individuals who had elevated serum LDL cholesterol and elevated plasma fibrinogen concentrations, there was a 6.1-fold increase in coronary risk. Conversely, individuals with low plasma fibrinogen had a lower incidence of coronary events even when serum LDL cholesterol was high.[71]

More recently, Maresca et al[58] conducted a meta-analysis including 13 prospective, 5 cross-sectional, and 4 case-control studies examining the association between plasma fibrinogen levels and cardiovascular disease and ischemic stroke. Among individuals with plasma fibrinogen levels in the highest tertile, the risk of cardiovascular events was 2× that of individuals with fibrinogen levels in the lowest tertile (OR, 1.99; 95% CI, 1.85 to 2.13). Fibrinogen levels are correlated with traditional vascular risk factors and outcomes, but whether this relationship is causal or the sequelae of systemic inflammation associated with atherosclerosis is unclear.

Factor VII

Factor VII (FVII) is a vitamin K–dependent zymogen that is converted to its active form FVIIa in the presence of tissue factor. The FVIIa/tissue factor complex converts factors IX and X into their active forms, leading to thrombin generation and fibrin clot formation. Plasma FVII levels are influenced by environmental and genetic factors and are positively correlated with triglycerides, BMI, oral contraceptive use, and postmenopausal status.[72] A number of prospective epidemiological studies have examined the relationship between FVII plasma levels and risk of CAD, yet it remains unclear whether these parameters affect risk. In the first NPHS, elevated FVII coagulant activity (FVII:C) was identified as an independent risk factor for fatal but not nonfatal MI in middle-aged males.[70] Subsequent studies, including a second NPHS (NPHSII), which was a prospective examination of hemostatic variables in the pathogenesis of CAD in healthy middle-aged men, were unable to confirm increased FVII:C as an independent predictor of CAD.[73–75]

Plasminogen Activator Inhibitor-1

The formation of a rigid clot is driven by thrombin and activated factor XIII and opposed by plasmin, which degrades cross-linked fibrin into FDP. The actions of plasmin are inhibited by α_2-antiplasmin, which rapidly binds plasmin in the circulation and is also incorporated into the developing clot. The generation of active plasmin requires cleavage of plasminogen by specific serine proteases: tissue plasminogen activator and urinary plasminogen activator, a process that is, in turn, inhibited by PAI-1.[76] Exposure to thrombin causes conversion of PAI-1 from a latent to an active form, which acts to stabilize clot formation.

PAI-1 gene transcription is induced by insulin, glucocorticoids, angiotensin II, very low–density lipoprotein, and acute-phase cytokines,[76–81] findings consistent with the observations that insulin resistance, dyslipidemias, and systemic inflammation are themselves associated with impaired fibrinolysis.[82,83] Investigators have also demonstrated that there is enhanced expression of PAI-1 in diseased vessels, and that expression is proportional to the degree of atherosclerotic burden.[84–86] In addition, PAI-1 may impair normal vascular remodeling through its effects on integrin expression and cellular migration,[87,88] findings that are supported by animal models in which overexpression of PAI-1 resulted in age-dependent coronary arterial thrombosis.[89]

The PAI-1 gene is located on chromosome 7 and has a single guanine bp insertion/deletion polymorphism in the promoter region at position −675, resulting in 5G and 4G alleles. Individuals who are homozygous for the 4G allele exhibit ≈25% higher PAI-1 plasma concentrations than their homozygous 5G counterparts.[90,91] The effect of the 4G/5G polymorphism on the risk of arterial events has been evaluated in several studies. Eriksson et al[91] reported that the OR for MI in a cohort of 94 young men was 2.15 (95% CI, 1.17 to 3.96) for individuals with the 4G/4G genotype compared with their 4G/5G and 5G/5G counterparts. In a larger cohort of older men in the US Physicians Health Study, Ridker et al[92] found no significant difference in the RR of first MI among patients with the 4G/4G genotype compared with controls. Similar negative findings have been reported in an elderly cohort of men and women.[93]

Studies in women have also been equivocal, with some even reporting a protective effect for carriership of the 4G allele. In a case-control study including 226 women 53 to 71 years of age, the 4G/5G genotype was associated with an adjusted OR of 1.6 (95% CI, 1.2 to 2.1) for the development of MI.[94] Conversely, in a cohort of 78 women <45 years of age with MI, carriership of the 4G allele was associated with a combined adjusted OR of 0.5 (95% CI, 0.29 to 0.85) when compared with the 5G homozygotes.[95] In a prospective cohort study of 498 women who died of cardiovascular disease and 512 controls, Roest et al[96] found no evidence that the 4G/5G genotype contributed to the risk of MI, and that the 4G allele was actually protective for cerebrovascular mortality with an OR of 0.4 (95% CI, 0.2 to 0.7) for the 4G/4G genotype and 0.7 (95% CI, 0.4 to 1.1) for the 4G/5G genotype when compared with 5G homozygotes.

Thogersen et al, in a prospective nested case-control study of a population with a high prevalence of CAD, reported that although levels of PAI-1 appeared to positively correlate with the incidence of first MI, this association was not statistically significant after adjustment for traditional cardiovascular risk factors (diabetes, smoking, hypertension, BMI, cholesterol, and apolipoprotein A-I).[97] These results are consistent with the findings of Juhan-Vague et al,[98] who reported that among a cohort of 3043 patients with angina pectoris, the prognostic role attributable to PAI-1 was no longer significant when adjusted for variables associated with insulin resistance. At present, there are no compelling epidemiological studies that define PAI-1 as a clear risk factor for arterial thrombotic events.

Hereditary Thrombophilias

The hereditary thrombophilias comprise a group of disorders that lead to an increased risk of venous thromboembolism. These include the factor V Leiden (G1691A) mutation, which results in activated protein C resistance, the prothrombin gene mutation (G20210A), which leads to elevated plasma pro-

thrombin levels, and quantitative or qualitative deficiencies in the natural anticoagulants protein C, protein S, and antithrombin. The importance of these genes in the pathogenesis of venous thromboembolism is well established,[99–103] but it is controversial whether these abnormalities contribute to arterial thrombotic events.

There have been a number of studies examining whether factor V Leiden, a prevalent abnormality in white populations, leads to an increased risk for arterial thrombotic events. In a cohort of healthy US male physicians >40 years of age in whom there was a low prevalence of smoking, no association was found between the factor V Leiden mutation and the risk of MI or stroke.[104] In a younger cohort of Italian patients with MI <45 years of age, Ardissino et al[105] found that the factor V Leiden mutation was not an independent risk factor for MI. Similarly, in a nested case-control study of data from the Cardiovascular Health Study, Cushman et al[106] examined 373 elderly patients with MI, angina, stroke, or transient ischemic attack and 482 controls; they found that even after adjusting for cardiac risk factors, heterozygosity for factor V Leiden was not associated with any of these end points.

Other investigators have found evidence that the factor V Leiden mutation contributes to the risk of arterial thrombotic events in specific populations. Data from a case-control study of MI in young women between 18 and 44 years of age indicate that heterozygosity for factor V Leiden is a risk factor but only among patients with other cardiac risk factors.[107] After adjustment for age, the presence of the factor V Leiden mutation was associated with a 2.4-fold increased risk of MI, but these data did not reach statistical significance (8 of 79 or 9.5% of patients with MI compared with 4.1% in controls). However, among individuals who had factor V Leiden and a smoking history, the OR was 32 (95% CI, 7.7 to 133) for MI. Interestingly, whereas other cardiac risk factors such as older age, obesity, hypercholesterolemia, hypertension, diabetes, family history of ischemic heart disease, and postmenopausal status (surgically induced) were associated with cardiac events, the use of oral contraceptives was not. Data from this cohort indicate that the prothrombin gene mutation is also a risk factor for MI (see below) but, again, only in current cigarette smokers.[108]

Investigators have also examined the contribution of the prothrombin gene mutation to the development of arterial thrombosis. Using data from the Physicians Health Study, Ridker et al[109] performed a prospective, nested case-control study to determine the impact of the prothrombin gene mutation on the risk of arterial thrombotic events. After correcting for hypertension, diabetes, BMI, and hyperlipidemia, the adjusted RRs for MI and stroke were 0.8 (95% CI, 0.4 to 1.4) and 1.1 (95% CI, 0.5 to 2.4), respectively. Other investigators have reported an association in subpopulations of patients with MI. In a case-control study of 79 women who had experienced an MI <44 years of age, Rosendaal et al[108] reported that the prothrombin gene mutation was present in 5.1% of patients and 1.6% of controls. This corresponded to an age-adjusted OR of 4.0 (95% CI, 1.1 to 15.1). When these data were further analyzed, it emerged that this overall increase in risk was actually attributable to the contribution of patients who had the prothrombin gene mutation as well as another cardiac risk factor. Among patients who had the prothrombin gene mutation and a history of smoking, the OR for MI was 43.3 (95% CI, 6.7 to 281); for patients with the prothrombin gene mutation and a metabolic risk factor (diabetes, obesity, or hyperlipidemia), the OR was 33.8 (95% CI, 5.5 to 209).

Kim and Becker[110] performed a meta-analysis of 56 case-control and cohort studies that examined the factor V Leiden, prothrombin G20210A, and MTHFR C677T mutations and the risk of arterial thrombotic events. In the studies examining the relationship between the factor V Leiden mutation and the end points of MI, ischemic stroke, and peripheral vascular disease, the summary ORs were 1.1 (95% CI, 0.88 to 1.36), 1.27 (95% CI, 0.86 to 1.87), and 0.91 (95% CI, 0.38 to 2.16), respectively. When the analysis was restricted to patients <55 years of age with factor V Leiden, the summary OR for the combined end points of MI, ischemic stroke, and peripheral vascular disease was 1.37 (95% CI, 0.96 to 1.97), and among women with factor V Leiden, the summary OR for all events was 1.79 (95% CI, 0.54 to 5.88). In the analysis of patients with the prothrombin G20210A mutation, the summary ORs were 1.28 (95% CI, 0.94 to 1.73) for MI, 1.30 (95% CI, 0.91 to 1.87) for ischemic stroke, and 0.26 (95% CI, 0.03 to 2.04) for peripheral vascular disease, the latter representing only 1 study. Among patients <55 years of age with the prothrombin G20210A mutation, the summary OR for the combined end points of MI, ischemic stroke, and peripheral vascular disease was 1.66 (95% CI, 1.13 to 2.46) and 1.73 (95% CI, 0.99 to 3.02) for women.

At present, there are no convincing data that other thrombophilic states such as deficiencies of protein C, protein S, or antithrombin confer an increased risk of arterial thrombosis, but evaluation of these associations is complicated by the relative infrequency of these defects in the general population.

Platelet Hyper-Reactivity

The degree to which differences in platelet reactivity or receptor affinity explain the variability in vascular events among individuals is an area of active investigation.

Mutations in the GpIIb and GpIIIa subunits may result in decreased receptor expression, altered receptor activation, or ligand affinity.[111] The most common polymorphism is the C1565T mutation in the IIIa subunit, which results in a wild-type allele designated PL^{A1} with leucine at amino acid 33 and a PL^{A2} allele with proline at position 33. Among individuals of European ancestry, the PL^{A1} and PL^{A2} alleles occur at a frequency of ≈85% and 15%, respectively.[112]

In a meta-analysis including 9095 patients and 12 508 controls, Di Castelnuovo et al[113] found that carriership of the PL^{A2} allele conferred an OR of 1.10 (95% CI, 1.03 to 1.18) for coronary outcomes and 1.21 (95% CI, 1.05 to 1.38) among individuals who were <60 years of age. More recently, the Atherosclerosis, Thrombosis, and Vascular Biology Italian Study Group[105] reported the results of a case-control study of 1210 patients who had experienced MI <45 years of age and 1210 controls. The OR for carriership of the PL^{A2} allele was 0.9 (95% CI, 0.8 to 1.2) compared with PL^{A1} homozygotes.

Relationship of Risk Factors and Arterial Thrombotic Events

Arterial Thrombotic Risk Factor	Associated With Disease	Modifiable
Elevated homocysteine	Established	Plasma levels can be reduced with vitamin therapy, but clinical effect has not been demonstrated.
Elevated CRP	Established	Plasma levels are modifiable with statin therapy with improved clinical outcomes
Presence of an LA	Likely, data limited	Not modifiable, but clear risk reduction with anticoagulation
Elevated levels of aCL	Possibly elevated IgG	Not modifiable, but clear risk reduction with anticoagulation
Elevated titers of ß2-GPI antibodies	Possibly ß2-GPI antibody– dependent LA	Not modifiable, but clear risk reduction with anticoagulation
Elevated fibrinogen	Established	Not modifiable, but clusters with other inflammatory markers and tobacco use
Elevated FVII	Not established	No
Elevated PAI-1	Not established	No
Factor V Leiden G1691A mutation	Possibly	Modest increase risk in combination with other cardiac risk factors, <55 years of age, and women
Prothrombin G20210A mutation	Possibly	Modest increase risk in combination with other cardiac risk factors, <55 years of age, and women
Protein C deficiency	Not established	No
Protein S deficiency	Not established	No
Antithrombin III deficiency	Not established	No
Platelet glycoprotein IIb/IIIa C1565T polymorphism	Not established	No
Platelet glycoprotein GpIb-IX-V C3550T	Not established	No
Platelet glycoprotein Ia/IIa C807T and G873A	Not established	No

Subsequent larger prospective studies have failed to demonstrate a relationship between the PL^{A2} allele and thrombotic outcomes. Ridker et al[114] examined the distribution of the PL^{A1}/PL^{A2} polymorphism among a subgroup of 374 individuals from the US Physician's Health Study who developed MI, stroke, or venous thrombosis and matched controls. They found no statistically significant difference in the RR of any vascular event among men homozygous or heterozygous for the PL^{A2} polymorphism when compared with men who were homozygous for the PL^{A1} allele. Analysis of data from the Etude Cas-Temoins sur l'Infarctus du Myocarde (ECTIM) database also failed to demonstrate an association between the PL^{A2} allele and vascular outcomes.[115] Based on the available literature, it would appear that the association of the PL^{A2} polymorphism with overall arterial vascular disease outcomes is minimal at most.

Several polymorphisms in the GpIb-IX-V platelet receptor have been studied with respect to their impact on arterial thrombotic outcomes. A C3550T mutation in the gene encoding the GpIbα subunit results in a threonine to methionine substitution at position 145 in the protein sequence. These variants display distinct antigenicity (the 145 M variant cosegregates with Ko(a) and Sib(b)[116]) and have been designated the threonine human platelet antigen (HPA)-2a and methionine HPA-2b polymorphisms. There is also a highly polymorphic region within the Ibα subunit which varies based on the number of variable number of tandem nucleotide repeats (VNTR) of a 13 amino acid sequence; these are designated D,C,B,A.[61]

Gonzales-Conejero et al[117] performed 3 case-control studies examining 104 patients with acute cerebrovascular accidents, 101 patients with CAD, and 95 patients with deep venous thrombosis. Among individuals with the VNTR C/B genotype, they found an OR of 2.84 (95% CI, 1.28 to 6.41) for CAD and an OR of 2.83 (95% CI, 1.16 to 7.07) for cerebrovascular accidents compared with the C/D and C/C genotypes, which constituted ≈80% to 90% of the cases and controls. There was a trend toward an association between the HPA-2b polymorphism and CAD (OR, 2.09), but this did not reach statistical significance. Although these data are supported by the results of several other case-control and postmortem studies,[118–120] there are multiple other studies that contradict these findings.[121–123]

Several polymorphisms in the α_2-subunit of the Gp Ia/IIa glycoprotein platelet receptor have been described; these include C807T in exon 7 and G873A in exon 8, which are in linkage disequilibrium and result in a higher receptor density than the wild-type receptor.[124] Santoso et al[125] tested for the C807T polymorphism in 2237 patients referred for diagnostic coronary angiography. They reported an OR of 1.57 (95% CI, 1.14 to 2.13; $P=0.004$) for individuals <62 years of age and an OR of 2.61 (95% CI, 1.26 to 5.41; $P=0.009$) for individuals <49 years of age. In 12 other case-control studies that examined the role of the C807T allele in the development of acute coronary syndromes, nonfatal MI, or CAD, only 3 have demonstrated an association between this allele, whereas 9 have not.[126] Investigators have also examined the C807T polymorphism and the risk of ischemic stroke, but these, too, have presented conflicting data.[126,127]

Conclusions

Arterial thrombosis is a complex phenomenon, involving a myriad of molecular, genetic, cellular, and environmental factors. The relationship between a number of potential risk

factors that may result in endothelial damage or altered hemostatic equilibrium and arterial thrombotic events are summarized in the Table. There are compelling data that elevated levels of plasma homocysteine, whether attributable to diet or one of several genetic mutations, are associated with arterial thrombosis. However, the mechanisms by which this occurs are uncertain, and to date, there is no evidence that this is a modifiable risk factor. CRP has also emerged as a significant hemostatic risk factor for arterial thrombotic events, an effect that is independent of and additive to traditional cardiac risk factors. However, the pathophysiological basis for this finding is unknown, and whereas statin therapy lowers CRP levels in a manner independent of its lipid-lowering effects, it remains to be seen whether this effect is primary or, rather, a reflection of other systemic immunomodulatory effects of these medications.

The increased risk of venous thrombosis among patients with the antiphospholipid antibody syndrome has led to considerable interest in the effects of these autoantibodies on the development of arterial thrombotic events. Unfortunately, this literature experiences a considerable lack of uniformity in entry criteria and assay selection as well as an inordinate reliance on retrospective and observational studies. These caveats notwithstanding, it appears that the risk of arterial thrombotic events is greatest for patients with the lupus anticoagulant, perhaps partially mediated by the β_2-GPI subtype and, to a lesser extent, elevated titers of anticardiolipin IgG.

Among the clotting factors for which elevated levels have been associated with an increased risk of arterial thrombosis, the data are consistent only for fibrinogen, particularly in the setting of other risk factors including smoking, male gender, and hyperlipidemia. However, it should be noted that although the relationship between genotype and fibrinogen level are reasonably well established, the literature connecting specific genotypes to clinical outcomes are contradictory.

Multiple polymorphisms in the FVII gene have been identified that have a predictable effect on plasma levels of FVII. However, the literature supporting a relationship between theses genotypes and arterial thrombotic outcomes have been inconsistent. Similarly, the results of studies on the PAI-1 4G/5G polymorphism have also been contradictory. In certain subsets of patients, including those with concomitant cardiac risk factors, <55 years of age, and women, carriership of the factor V Leiden or the prothrombin G20210A mutation is associated with an increased risk of arterial thrombotic events. Finally, although numerous polymorphisms in the platelet receptor glycoproteins IIb/IIIa, IX-V, and Ia/IIa have been identified, their relationship to the development of arterial thrombotic events remains unproven.

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