Thrombogenic Risk Factors for Atherothrombosis

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Thrombosis superimposed on a disrupted plaque is the proximate event that triggers most acute ischemic syndromes and episodes of sudden cardiac death. A significant number of acute ischemic events occur in individuals without traditional atherosclerosis-related risk factors. In an attempt to pinpoint additional risk factors, researchers are examining the thrombotic cascade and the cellular components, plasma proteins, and endothelium-derived mediators, as well as their genetic polymorphisms, that may affect this system. This article enumerates a number of potential hemostatic risk factors and discusses the evidence linking them to atherothrombotic events.
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Atherosclerosis predisposes to arterial thrombosis and thrombosis superimposed on a disrupted (ruptured or eroded) plaque is the proximate event that triggers most acute ischemic syndromes and episodes of sudden cardiac death.[1] Although a number of risk factors for atherothrombosis have been defined through observational and epidemiologic studies, a significant number of acute ischemic events occur in individuals without traditional atherosclerosis-related risk factors.[2]

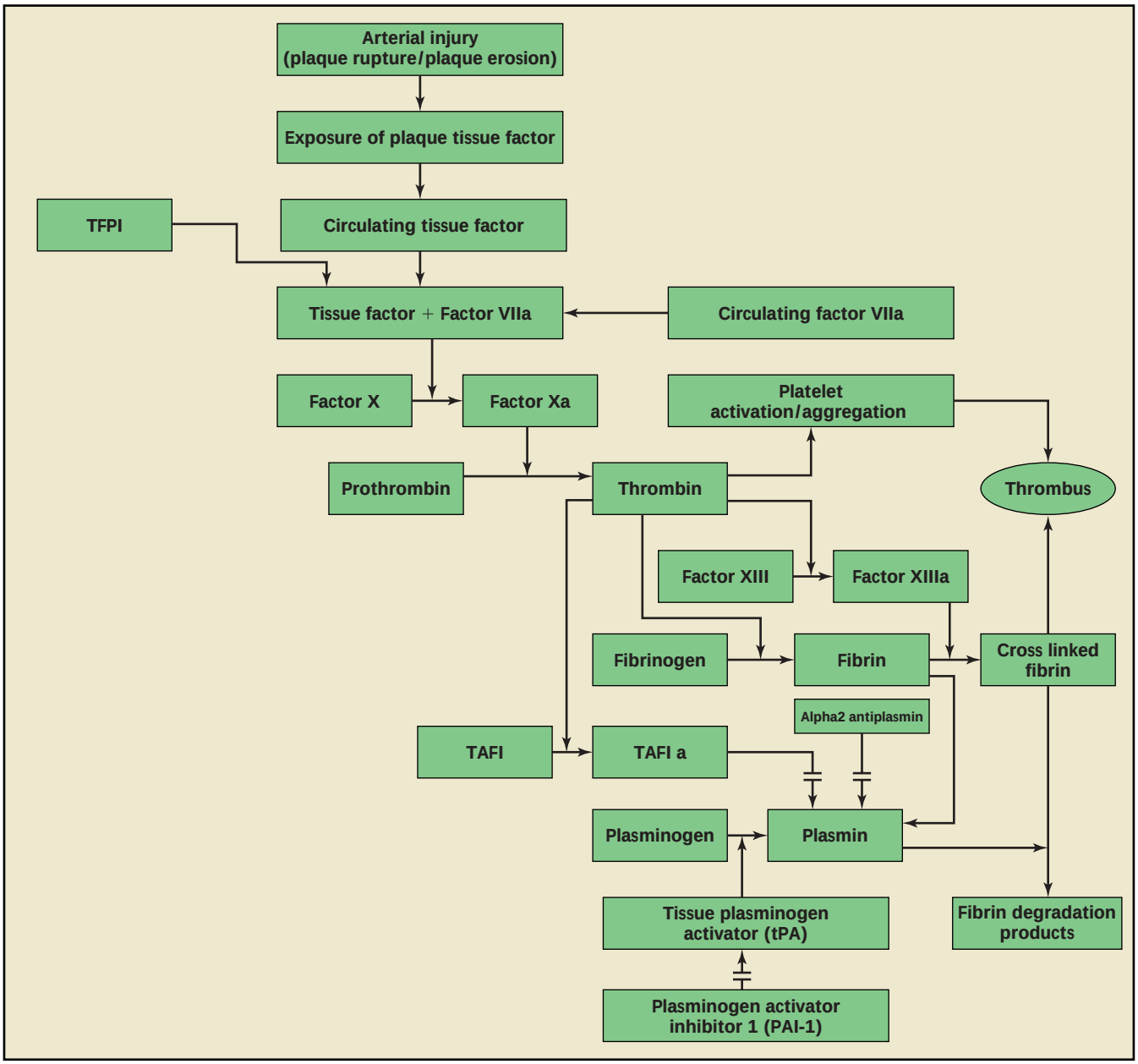

Figure 1. *This schematic illustrates an abbreviated version of the thrombotic cascade initiated by arterial injury. TFPI, tissue factor pathway inhibitor; TAFI, thrombin activated fibrinolysis inhibitor.*

More than 100 years ago, thrombosis was postulated to result from a triad of factors (known as Virchow's triad) consisting of stasis, injury to the vessel wall, and circulating thrombogenicity. Virchow's triad appears to be particularly relevant for venous thrombosis, whereas arterial thrombosis has generally been thought to be related largely to arterial injury (endothelial erosions and plaque rupture) and perhaps aided and abetted by abnormal flow patterns resulting from luminal stenosis. A number of studies have also attempted to highlight the potential role of circulating thrombogenicity in arterial thrombosis.[3-7] Normal hemostasis depends on a closely regulated interaction between prothrombotic and antithrombotic/thrombolytic processes mediated by cellular components, soluble plasma proteins, and endothelium-derived mediators (Figure 1).

Exposure of the vessel wall to subendothelium leads to platelet adhesion to collagen and von Willebrand factor, followed by platelet activation and platelet aggregation.

Platelet aggregation is mediated by crosslinking of adjacent platelets through fibrinogen and other adhesive proteins, utilizing the platelet glycoprotein IIb/IIIa receptors. Exposure of blood to tissue factor-rich atherosclerotic plaque also results in activation of the clotting cascade with local generation of thrombin, through a series of steps. Thrombin activates platelets via protease-activated receptors 1 and 4. Exposure of negatively charged phospholipids on the platelet exterior provides a surface on which clotting proteins assemble. Thrombin also converts fibrinogen to fibrin, and the resulting fibrin polymers are cross-linked and stabilized by Factor XIII, a transglutaminase activated by thrombin. Several endogenous inhibitors of clotting regulate the clotting process and include antithrombin, protein C, and others. Fibrin, in turn, is lysed by plasmin, which is generated from its precursor, plasminogen, via tissue type plasminogen activator (t-PA). Fibrinolysis, in turn, is inhibited by t-PA inhibitor, plasminogen activator inhibitor-1 (PAI-1), alpha-2 antiplasmin, which inhibits free plasmin, and thrombin-activated fibrinolysis inhibitor. Recently, it has also been suggested that circulating forms of tissue factor derived from circulating activated leukocytes, platelets, and procoagulant shed microparticles (derived from endothelial cells, platelets, and leukocytes) or a soluble variant of tissue factor lacking the transmembrane domain that may also contribute to thrombin generation and clotting. Genetic or acquired abnormalities that change the production, activity, bioavailability, or metabolism of specific hemostatic factors could alter this delicate balance, creating a circulating prothrombotic milieu, which predisposes to arterial thrombosis.

Platelets and Atherothrombosis

Platelet adhesion and aggregation at the site of arterial injury play a critical role in thrombus formation. Platelets are activated by contact with collagen, shear stress, and mediators including thrombin and platelet surface receptors, particularly the glycoprotein IIb/IIIa receptors (also known as integrin alpha IIb beta 3), which bind fibrinogen and von Willebrand factor, thereby playing a seminal role in platelet-to-platelet interaction and platelet aggregation. Increased platelet volume and spontaneous aggregation have been suggested to increase the risk of recurrent myocardial infarction.[8,9] Several genetic polymorphisms of this receptor complex have been described.[3] A common polymorphism involves a Leucine to Proline substitution at position 33 in exon 2 of the GPIIIa gene, which produces a conformational change in the amino-terminal disulfide loop, critical for fibrinogen binding. This polymorphism, known as the PL^{A2}, is present in 15% of whites, 5% to 8% of blacks, but rarely if ever occurs in Asians. It is thought to heighten platelet responsiveness to agonists and has been linked to a markedly increased risk for coronary heart disease among subjects less than 60 years of age, especially among smokers. However, these findings have not been consistently substantiated in larger studies after accounting for confounding variables.[10-20] Polymorphisms involving other platelet glycoprotein-receptor genes such as those in the GP Ia/IIa (also known as integrin alpha 2 beta I) receptor, which acts as the main adhesion receptor for von Willebrand factor, have also yielded inconsistent results.[3]

Clotting Factors and Atherothrombosis

Fibrinogen

Fibrinogen is a major clotting protein, not only as the precursor of fibrin but also in terms of the important role it plays in plasma viscosity and platelet aggregation. Plasma fibrinogen levels are strongly correlated with traditional vascular risk factors such as age, physical inactivity, hypertension, smoking, and features of the insulin resistance syndrome. Further, fibrinogen is an acute-phase reactant. The acute-phase responses arising from viral infection, inflammatory stimuli, and smoking have all been implicated in the development of atherothrombosis. Several prospective studies, including the Northwick Park Heart study, the Prospective Cardiovascular Munster (PROCAM) study, and the Prospective Epidemiological Study of Myocardial Infarction (PRIME), have identified an association between higher plasma fibrinogen levels and the risk of myocardial infarction, stroke, and peripheral vascular disease, with an independent relative risk of arterial disease around 2 to 2.5 in the highest compared with the lowest fibrinogen quartile.[21-23] Several genetic polymorphisms of the fibrinogen gene, which influence circulating fibrinogen levels as well as other functional aspects of clot formation, have been identified.

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However, the attempts to link these polymorphisms to atherothrombotic vascular disease have, in general, yielded weak or inconsistent results.[3] A recent meta-analysis of fibrinogen levels among 154,211 participants from 31 studies reported 6944 first non-fatal acute myocardial infarction or stroke events and 13,210 deaths with cause-specific mortality data noted.[24] The age- and sex-adjusted hazard ratio per 1 gram increase in the usual level of fibrinogen was 2.42 (95% CI: 2.24-2.60) for coronary heart disease, 2.06 (95% CI: 1.83-2.33) for stroke, 2.76 (95% CI: 2.28-3.35) for vascular mortality, and 2.03 (95% CI: 1.90-2.18) for nonvascular mortality.[11] The hazard ratios, after adjustment for established risk factors, were reduced to 1.8 for coronary heart disease and stroke. Thus a large body of data suggests a moderately strong independent relationship between fibrinogen levels and cardiovascular risk. Although biologically plausible explanations could account for a causal relationship between fibrinogen levels and atherothrombosis, it is also possible that elevated fibrinogen levels reflect the inflammation associated with atherosclerosis.[24]

Clotting Factor VII

Factor VII (FVII) is a vitamin K-dependent coagulation factor and its levels are influenced by age, body mass index, and plasma triglyceride levels.[3] Several prospective studies have examined the relationship between FVII-mediated procoagulant activity (FVIIc) and atherothrombosis but the results have been inconsistent. The Northwick Park Heart study found a significant association between FVIIc and coronary heart disease that was stronger than that with cholesterol levels.[21] However, subsequent reports, taking into account confounding variables, failed to confirm this finding.[25] Similarly, several genetic polymorphisms of the FVII gene that influence its levels have been described but the relationship of these polymorphisms to coronary heart disease has again been shown to be weak, negative, or inconsistent.[3,26-33]

Clotting Factor XIII

Clotting factor XIII is involved in stabilizing a fibrin clot. Several genetic polymorphisms have been identified in the Factor XIII gene but once again no consistent relationship of these polymorphisms to atherothrombotic vascular disease has been confirmed; in fact, some of the polymorphisms have actually shown an inverse relationship.[34-40]

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Clotting Factor V/Prothrombin

Several studies have evaluated the relationship between genetic abnormalities in the Factor V and prothrombin genes and risk of arterial thrombosis. Positive associations have in general been observed in studies involving highly selected populations or among children or have factored in interactions with environmental risk factors.[41,42] Thus Factor V Leiden mutation was shown to be associated with a 2.5-fold increased risk of non-fatal myocardial infarction among young women, predominantly smokers.[43] Similar findings were noted among carriers of the prothrombin *20210A* allele, who had a 4-fold increase in the risk of myocardial infarction that was again increased more than 40-fold among smokers.[44] A combined analysis of Factor V Leiden and the prothrombin *20210A* allele in this population showed that the effect of major coronary risk factors was increased 4- to 6-fold by the presence of one of these inherited, prothrombotic risk factors. Subsequently, 2 case-control studies of men showed an increased risk of myocardial infarction associated with Factor V Leiden and the prothrombin 20210G/A mutations, predominantly in the presence of other cardiovascular risk factors.[45,46]

Notwithstanding these positive associations, most studies examining the association of Factor V Leiden (1691G/A) mutation and prothrombin 20210G/A mutation to arterial thrombotic disease have been negative, even among young subjects.[3]

Thrombomodulin

Thrombomodulin is an endothelial cell-surface receptor for thrombin that accelerates thrombin-induced activation of the natural anticoagulant protein C. Reduced plasma thrombomodulin levels were associated with an increased risk of myocardial infarction in a prospective case-control study.[47] The association of certain genetic polymorphisms in the thrombomodulin gene with atherothrombotic disease has, however, been inconsistent.[48,49]

Endothelium-Derived Fibrinolysis-Related Mediators in Atherothrombosis

Endogenous clot lysis is dominantly mediated by endothelium-derived, tissue-type plasminogen activator

(t-PA), the action of which is, in turn, opposed by its inhibitor, known as the plasminogen activator inhibitor-1 (PAI-1). Circulating t-PA is most closely associated with PAI-1 as a complex, and thus circulating levels of t-PA do not necessarily reflect functionally active plasminogen activator levels.

Elevated levels of both t-PA and PAI-1 have been associated with an increased risk of arterial thrombotic disease in some, but not all, relevant studies.[25] An imbalance of this fibrinolytic equilibrium is encountered primarily in the insulin resistance syndrome and hypertriglyceridemia (elevated triglycerides are associated with increased PAI-1 levels), which leads to increased plasma PAI-1 and t-PA antigen levels (reflecting inactive t-PA/PAI-1 complexes) with a consequent decrease in fibrinolytic activity.[50] Genetic polymorphisms of t-PA and PAI-1 genes have not demonstrated a uniform relationship to atherothrombotic vascular disease.[3] The most common genetic polymorphism of PAI-1 involves a 4G/5G insertion/deletion polymorphism at −675 position of the promoter of PAI-1 gene with the 4G allele conferring increased responsiveness to triglycerides. A recent meta-analysis of 9 studies with over 1500 patients showed only a weak positive association between 4G allele and risk of myocardial infarction, despite earlier case-control studies, which had suggested a stronger link.

A recently identified inhibitor of fibrinolysis is a plasma carboxypeptidase called thrombin-activatable fibrinolysis inhibitor (TAFI).[51] Plasma TAFI concentrations demonstrate high interindividual variability that is poorly explained by environmental factors.[52] Activation of TAFI occurs through the thrombin-thrombomodulin complex and results in prolongation of clot lysis time. Increased plasma TAFI levels have been associated with an increased risk of both deep-vein thrombosis and symptomatic or angiographic coronary artery disease in some studies, whereas others have shown an inverse relationship to risk of myocardial infarction.[53-55] In the past few years, several polymorphisms that have been described in the TAFI gene have been identified as having an inconsistent relationship to atherothrombotic risk.[3]

Endothelium-derived nitric oxide, produced by the action of endothelial nitric oxide synthase (E-NOS) from Arginine, exerts antithrombotic actions in addition to its anti-inflammatory, anti-oxidant, vasodilator and anti-proliferative effects.[56] Several genetic polymorphisms of the eNOS gene have been identified but their relationship to atherothrombosis has been inconsistent at best.[3]

Leukocyte Count and Atherothrombosis

Inflammation is critically linked to various pathophysiologic events leading to initiation, progression, and destabilization of atherosclerosis.[1] A number of epidemiologic studies have shown an association between elevated leukocyte count and coronary heart disease.[57-65]

Main Points

- Atherosclerosis predisposes to arterial thrombosis and thrombosis superimposed on a disrupted (ruptured or eroded) plaque is the proximate event that triggers most acute ischemic syndromes and episodes of sudden cardiac death.
- Platelet adhesion and aggregation at the site of arterial injury play a critical role in thrombus formation.
- Polymorphisms involving platelet glycoprotein-receptor genes, such as those in the GP Ia/IIa and GP IIb/IIIa receptors, have yielded inconsistent results when tested for linkage to increased atherosclerosis and thrombosis.
- Several prospective studies, including the PROCAM study and PRIME, have identified an association between higher plasma fibrinogen levels and the risk of myocardial infarction, stroke, and peripheral vascular disease. However, although several genetic polymorphisms of the fibrinogen gene, which influence circulating fibrinogen levels as well as other functional aspects of clot formation, have been identified, attempts to link these polymorphisms to atherothrombotic vascular disease have yielded inconsistent results.
- Other clotting proteins including clotting factors VII, XIII, and V/prothrombin have been identified as prothrombotic but neither these proteins nor their identified polymorphisms have been consistently linked to increased atherothrombotic events.
- Endogenous clot lysis is dominantly mediated by tissue-type plasminogen activator (t-PA), the action of which is, in turn, opposed by its inhibitor, known as the plasminogen activator inhibitor-1 (PAI-1). Elevated levels of both t-PA and PAI-1 have been associated with an increased risk of arterial thrombotic disease in some studies. Genetic polymorphisms of t-PA and PAI-1 genes have not demonstrated a uniform relationship to atherothrombotic vascular disease.

Similarly, in acute coronary syndromes, elevated leukocyte count has been linked to increased risk for fatal and nonfatal cardiovascular events.[66-69] A recent study suggested that the cardiovascular risk of elevated white blood cell counts is carried by increased circulating neutrophil counts and decreased total mononuclear cell counts (lymphocytes plus monocytes).[70,71] However, some studies have failed to confirm these relationships after correction for confounding risk factors such as smoking.[27,70]

Conclusion

Despite the known importance of arterial thrombosis as the major complication of atherosclerosis, a circulating thrombogenic state, as measured by plasma factors and their genetic variants, has not been consistently shown to be strongly linked to atherothrombotic events, with the possible exception of plasma fibrinogen levels. These observations continue to suggest that the predominant factors contributing to arterial thrombosis in atherosclerosis have to do with disruption of the plaque itself (plaque rupture and superficial endothelial erosions). Although local flow conditions and circulating milieu may regulate the magnitude of the thrombotic response, a strong impact of such factors is unlikely in most instances. ■

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