



CLINICAL HEMOSTASIS REVIEW

An Update on Advances and Issues in Hemostasis

Hemostatic Factors in the Development of Arterial Thrombosis

By Dorothy M. Adcock, MD

INTRODUCTION

Thrombus development represents a complex interaction between the blood vessel wall, coagulation proteins, and platelets. Thrombosis is a frequent event that can develop in either the arterial or venous circulation. Arterial thrombosis, in fact, is the most common cause of death in North America as it is the etiology of most acute myocardial infarctions (MI) and cerebral vascular accidents. These entities represent the two most common forms of cardiovascular disease. Venous thromboembolic disease is the third most common form of cardiovascular disease in North America and typically presents as deep venous thrombosis and pulmonary embolus.

PATHOPHYSIOLOGY

There are significant differences in the pathology of blood clots between the venous and arterial systems. The venous circulation is a low flow, low pressure system. Clots that develop in the venous system are generally relatively large in size and are composed predominantly of fibrin enmeshed with cellular components. Stasis and changes in blood composition that induce hypercoagulability, such as the fac-

tor V Leiden polymorphism and antithrombin deficiency represent the most important contributors to clot formation in the venous system. Venous thrombosis may in fact, occur spontaneously in individuals with genetic abnormalities associated with hypercoagulability.

Conversely, the arterial system is a high flow, high pressure system. Platelets are a vital component of arterial thrombi but development of occlusive thrombi is also dependent on activation of the coagulation system. Occlusive clots in the arterial system are relatively rich in fibrin. Unlike the venous system, arterial thrombosis typically occurs in a setting of an underlying vascular abnormality, most commonly atherosclerotic vascular disease (ASVD). In the arterial circulation, vascular injury is the most frequent cause of clot development and thrombosis typically occurs in a setting of atheromatous plaque rupture or endothelial cell damage.

CONTRIBUTING FACTORS

The development of atherosclerotic vascular disease and associated arterial thrombosis is a complex, multi-genic disorder. Arterial thrombosis in the setting of

Objective: The reader will be able to discuss the alterations of hemostatic parameters associated with the development of arterial thrombosis.

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atherosclerosis is the culmination of interactions between gene-gene, gene-environment, and environment-environment influences where both genetic and acquired risk factors interact in an additive or synergistic manner. Well-established environmental and genetic risk factors for the development of arterial vascular disease include hyperlipidemia, obesity, cigarette smoking, diabetes mellitus, hypertension, hyperhomocysteinemia, and a positive family history of arterial vascular disease. Approximately 30% of arterial thrombotic events, however, occur in the absence of these traditional cardiovascular risk factors, suggesting the presence of additional, and to-date undefined, risk factors for the development of arterial thrombosis.

The multifactorial basis of arterial disease and the interactions among risk factors makes evaluation of underlying isolated genetic abnormalities a formidable task. Further compounding this issue is the observation that presence of an isolated polymorphism of a particular protein has little overall effect on its plasma levels, varying concentration by only about 5%. Therefore the effect of a single polymorphism may be difficult if not impossible to discern in isolation. According to Merlini and Ardissimo, the effect of any single genetic risk factor on arterial thrombosis is likely to be modest (a relative risk of 1.2 or 1.5). The effect of a single genetic risk factor, therefore may be overshadowed by the presence of more classic, (life style or environmental), risk factors. Furthermore, the overall risk associated with a single genetic abnormality is likely dependent on its interaction with other genetic risk factors or environmental factors that may be present and in fact, the interaction of these multiple risk factors may be necessary for disease expression. For example, risk associated with a specific polymorphism may be expressed only in the presence of significant underlying vascular disease or only in the presence of other polymorphisms. It may be more informative therefore to study the impact of multiple genetic risk factors on a cohort although this greatly complicates the establishment of an appropriate and well controlled study group. Given the complex, multi-genic basis of arterial disease and the interaction of

environmental and genetic factors, it is difficult to conduct controlled studies that account for all of the necessary variables such as; an adequate number of individuals studied, the ethnicity of the cohort, the prevalence of underlying vascular disease, the type of risk factors present, and the frequency of the allele being evaluated. Sample size is critical in properly assigning risk associated with a specific genetic factor and the sample size needed is largely dependent on the frequency of the allele being investigated. Many published studies are contradictory regarding the relative risk of the various risk factors described and the relationship between genetic risk factors of the hemostatic system and arterial thrombosis is not clear-cut.

In the evaluation of hemostatic factors and arterial thrombotic risk, investigators have focused on the various components of the hemostatic system including blood coagulation factors, coagulation inhibitors, fibrinolytic proteins, and platelet membrane receptors. This article will briefly review hemostatic risk factors in the development of arterial thrombosis.

The relationship of well-established risk factors for venous thrombotic disease, such as deficiencies of the naturally occurring anticoagulants (antithrombin, protein C and protein S) and polymorphisms in coagulation factors (Factor V Leiden, prothrombin G20210A), to the risk of developing arterial thrombosis has been extensively investigated. It is generally agreed that the relationship among venous risk factors and arterial thrombosis is not obvious. Studies suggest that the venous thrombophilic factors are not major risk factors for arterial thrombosis but may play a role in certain patient subgroups. For example, factor V Leiden and prothrombin G20210A polymorphisms do not confer an increased risk of arterial disease unless there are additional underlying arterial risk factors such as cigarette smoking. The well-established risk factors for venous thrombosis however, may contribute to arterial disease that develops in certain subgroups such as children with arterial stroke or pre-menopausal women who suffer MI.

There are very few well-established risk factors that are associated

- Hyperhomocysteinemia
- Dysfibrinogenemia
- Anti-phospholipid Antibodies
- Lupus Anticoagulant

TABLE 1. Well-established Risk Factors Associated with Both Venous and Arterial Thrombotic Disease.

with an increased risk of both venous and arterial thrombotic disease. These factors include protein-phospholipid complex antibodies (anti-phospholipid antibodies), rare cases of dysfibrinogenemia, and hyperhomocysteinemia.

Antibodies to protein-phospholipid complexes (lupus anticoagulants and antiphospholipid or anticardiolipin antibodies) are an important risk factor for the development of arterial thrombotic disease. Lupus anticoagulants are antibodies that interfere with phospholipid dependent coagulation assays and can be detected using a variety of tests such as the activated partial thromboplastin time, dilute Russell's viper venom time, and kaolin clotting time. Confirmatory assays typically include addition of excess phospholipid to neutralize the antibody. ELISA based assays can be used to detect protein phospholipid complexes and include the following assays; anticardiolipin antibody, anti-phosphatidyl serine, anti-prothrombin, anti-annexin V, and anti B2 Glycoprotein 1. Antibodies to protein phospholipid complexes are common with an incidence of 1% to 2% in the general population. Antibodies that are persistently elevated over at least a 6 to 8 week time period are more clinically significant than transient antibodies that may occur, for ex-

- Antithrombin Deficiency
- Protein C Deficiency
- Protein S Deficiency
- Activated Protein C Resistance
- Factor V Leiden
- Prothrombin G20210A
- Hyperhomocysteinemia
- Anti-phospholipid Antibodies
- Dysfibrinogenemia

TABLE 2. Well-established Risk Factors Associated with Venous Thrombotic Disease.

ample, secondary to infection. Persistent antiphospholipid antibodies are an important cause of MI and stroke, particularly when arterial thrombosis occurs at a young age or in individuals without evidence of traditional cardiovascular risk factors. Antiphospholipid antibodies can be seen in 7%

to 10% of unselected stroke patients and this association is greater in young adults and children. These antibodies have also been reported in up to 20% of individuals suffering MI who are less than 45 years of age.

Elevated fibrinogen activity levels (in the highest tertile) are associated with a two fold increased risk of myocardial infarction in both healthy and high risk individuals. This increased risk is independent of other well known risk factors. Fibrinogen levels elevate as an acute phase response. Levels likely also have a genetic basis in part and may be related to polymorphisms such as *BclI*, Thr313Ala, and 455G/A. Increased fibrinogen concentration may contribute to thrombotic risk by increasing blood viscosity, increasing fibrin formation, and enhancing platelet aggregation. In a recent trial, the administration of Bezafibrate, an agent that lowers fibrinogen levels and alters lipid profiles, was not associated with a reduction in the incidence of arterial thrombotic events. A direct cause-and-effect relationship between fibrinogen levels and arterial thrombotic disorders is therefore suspect.

Certain forms of dysfibrinogenemia may be a rare cause of arterial thrombosis. Arterial thrombosis can occur when the abnormal fibrinogen molecule is converted to fibrin clot that is relatively resistant to plasmin degradation. Dysfibrinogenemia is suspected in the presence of an elevated thrombin clotting time and reptilase

time. It is usually associated with normal or slightly decreased fibrinogen antigen levels and decreased fibrinogen activity.

Increased concentrations of Factor VIII and von Willebrand factor antigen are associated with an increased risk of arterial thrombotic events. Both proteins are acute phase reactants. Because these proteins circulate as a complex, levels generally correlate with one another. Although sustained elevations may have a genetic basis, factor VIII or von Willebrand gene polymorphisms that increase risk for arterial disease have not been described. The basis for increased clot development in patients with elevated concentrations of factor VIII is thought to be due to increase activation of thrombin activatable fibrinolysis inhibitor (TAFI) which leads to enhanced clot stability through diminished plasmin generation. Elevations of von Willebrand protein may induce thrombosis by enhancing the binding of platelets to the vasculature or possibly to each other.

In the Northwick Park Heart Study, elevations of factor VII activity were strongly associated with an increased risk of coronary events. This finding has not been supported in other studies and factor VIII levels have not been identified as an independent risk factor for arterial disease. Variations in assay methodology and reagent sensitivity may be one reason for discrepancies in study results. To date, five polymorphisms in the factor VII gene associated with increased factor VIII levels have been identified, but the relationship between these polymorphisms and arterial risk is unclear at this time.

Factor XIII functions as a transglutaminase and acts to covalently cross-link fibrin therefore stabilizing the fibrin clot. A polymorphism of the factor XIII gene, specifically val34leu, is reported to have a protective effect against the development of arterial events including myocardial infarction and stroke. This effect is paradoxical as the val34leu polymorphism enhances the transglutaminase activity of factor XIII, promoting clot stability and is associated with higher than normal levels of plasminogen activator inhibitor-1 (PAI-1).

Increased plasma concentrations of PAI-1 are consistently associated with

- Increased Fibrinogen Activity
 - Fibrinogen β chain Bc/I
 - Fibrinogen β chain -455G/A
 - Fibrinogen α Thr313Ala
- Increased Factor VII/von Willebrand Factor Complex Activity
- Increased Factor VII Activity
 - Factor VII HVR4
 - Factor VII -402G/A
 - Factor VII -401G/T
 - Factor VII -323 O/10
- Factor XIII Val34Leu
- Increased Plasminogen Activator Inhibitor-1
 - PAI-1 -675 4G/5G
- Increased Lipoprotein (a)
- Increased Homocysteine
 - MTHFR C677T
 - MTHFR A1298C
- Platelet Surface Glycoprotein Polymorphisms
 - Gp IIIa Pro33Leu (HPA-1b or PLA2)
 - Gp Ia VNTR
 - Gp Ia/IIa C807T

TABLE 1. Well-established Risk Factors Associated with Arterial Thrombotic Disease.

an increased risk of arterial disease including myocardial infarction and unstable angina. Elevated PAI-1 levels cause a decreased half life of tissue plasminogen activator (tPA) and therefore decreased potential for clot lysis. Elevations of PAI-1 can be in part genetically based although environmental factors such as insulin resistance and hypertriglyceridemia likely have a greater influence on PAI-1 levels.

The 4G insertion polymorphism of the PAI-1 gene is associated with increased PAI-1 levels due to increased mRNA. Although controversial, a meta-analysis suggests that the 4G allele is associated with a mild increased risk for cardiovascular events compared to the wild type (5G/5G). Studies have shown that subjects who are homozygous for the 4G allele have plasma PAI-1 concentrations approximately 25% higher than those with the 5G allele. Similarly, among patients with hypertriglyceridemia, those with the 4G allele

presence of hypertriglyceridemia.

Elevated lipoprotein a [Lp(a)] levels are associated with a risk of future arterial events. Lp(a) is composed of LDL particles linked to an apoprotein. This apoprotein is structurally similar to plasminogen and inhibits plasminogen binding to fibrin thereby inhibiting fibrinolysis. Oxidized Lp(a) may promote cholesterol deposition in the vessel wall enhancing the development of atherosclerotic vascular disease. Levels of Lp(a) are determined largely by genetic factors with little impact secondary to dietary modifications, lipid lowering drugs, and physical activity. Lp(a) levels increase following an acute arterial event and it is not known whether elevated Lp(a) levels are causative of arterial thrombosis or whether they represent a consequence of cardiovascular disease. Studies suggest that overall, effect of Lp(a) is probably small and evident only in that subgroup with the highest Lp(a) levels.

also have higher plasma PAI-1 concentrations than those with the 5G allele. There is still conflicting data on the strength of the relationship between PAI-1 gene polymorphism and MI, but it is suggested that the 4G allele is more likely to contribute to MI, particularly in the

Homocysteine is a precursor for the sulphur containing amino acid methionine. Levels can be increased in individuals with specific enzyme deficiencies or polymorphisms, with certain vitamin deficiencies, with renal failure, and as an effect of certain medications. Levels may also increase following acute arterial thrombosis making evaluation of the relationship with arterial disease difficult. Prospective studies investigating the risk of arterial thrombosis and homocysteine levels are inconsistent. Further studies to evaluate the effect of plasma homocysteine as well as the common polymorphisms that can contribute to increased homocysteine levels are needed.

Polymorphisms in the tissue factor pathway inhibitor gene, thrombomodulin, and epithelial protein C receptor genes may potentially confer increased risk for arterial disease. Studies to date are limited with inconsistent results and further investigation of these polymorphisms is needed.

Because platelets play an important part in arterial thrombosis it is important to consider the role adhesion molecule of the platelet surface play in increasing arterial risk. There are a number of glycoproteins on the surface of the platelet that function to bind proteins that either cause platelets to adhere to the site of vascular injury or aggregate to one another. Platelet membrane glycoproteins are highly polymorphic and allelic variations of the two major membrane adhesive receptors (GPIIb-IIIa and GPIb-IX) are the most commonly found.

One of the best-studied platelet glycoprotein polymorphisms is the PI^{A1} which is expressed on glycoprotein IIb/IIIa. This is the receptor involved in fibrinogen binding and platelet aggregation. A common polymorphism, seen in up to 15% of Caucasians and in 25% of individuals with Northern European heritage, is associated with a proline substitution for leucine at position 33 which is called PI^{A2} or HPA-2. It was initially reported that this polymorphism is associated with a 2 to 3 fold increased risk of myocardial infarction and this risk increases to 6 fold in those less than 60 years of age. Since the initial study was reported, a number of papers have been published which both support and refute the association

between P1A² or HPA-2 and arterial thrombosis.

The GPIIb-IX glycoprotein complex binds von Willebrand factor and therefore this complex plays an important role in the adhesion of platelets to regions of vascular injury. A number of polymorphisms of the GPIIb-IX complex have been described. The "length" polymorphisms include 4 different alleles and is called "length" because the alleles vary by the number of repeat base pairs inserted (A [four copies of the repeat], B [three copies], C [two copies], and D [one copy]) and consists of a variable number of tandem repeats (VNTR) of 39 base pairs. The B and D alleles have been associated with an increased risk of arterial thrombosis in small studies to date.

Glycoprotein Ia/IIa is the major platelet collagen receptor and is responsible for platelet adherence to exposed vascular subendothelium. There are two single nucleotide polymorphisms C807T and G873A that are in complete linkage disequilibrium (these polymorphisms are found in association more often than chance alone would predict) with one another. The C807T allele is associated with increased collagen receptor levels and increased collagen induced platelet adhesion. Preliminary results suggest that the C807T variant of glycoprotein Ia may be a genetic risk factor for early-onset arterial thrombotic disease.

CONCLUSION

In general, screening for arterial thrombotic risk factors is of value and can be recommended only if the identification of an abnormal result would have prognostic or therapeutic consequences. Screening for arterial thrombosis is best accomplished by evaluating for the traditional cardiovascular markers, such as diabetes mellitus, smoking, hypertension, and hypertriglyceridemia. Evaluation of venous thrombotic risk factors and especially antiphospholipid antibodies, hyperhomocysteinemia, and dysfibrinogenemia may be of value in children, young individuals or pre-menopausal women with arterial thrombosis, or in any individual with arterial disease in the absence of typical cardiovascular risk factors. Many of the hemostatic risk factors described in this article are inconclusive in their relationship with ar-

terial disease or have only a small influence on the development of arterial thrombosis. Further epidemiologic studies are needed to better determine the relationship of hemostatic risk factors and arterial thrombosis. ▲

KEYWORDS: *hormone replacement therapy, oral contraceptives, estrogen, thrombosis, postmenopausal*

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