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HYPERCOAGULABLE STATES: CHALLENGES AND OPPORTUNITIES

RALPH L. NACHMAN

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INTRODUCTION

Hypercoagulable states constitute a group of inherited as well as acquired clinical disorders that predispose patients to thromboembolic events. Most commonly, multiple factors acting together at a given point in time and place lead to the inappropriate generation of thrombin. In the last several decades a veritable explosion of new biologic information has greatly amplified our understanding of the blood coagulation system and the highly regulated mechanisms that govern thrombin generation at sites of vascular injury. In particular, delineation of a number of newly recognized inherited human thrombophilic states as well as the development of targeted deletion of specific genes in "knock out" mice has emphasized the extraordinary importance of the physiologic anticoagulant systems in maintaining blood fluidity (1,2,3).

Coagulation Physiology (Figure 1)

Coagulation is a highly regulated cascade of surface associated complexes of enzymes and cofactors and is initiated in response to trauma (physical as well as biochemical) by exposure of tissue factor, a cellular membrane protein that binds factor VIIa as well as the zymogen factor VII. The tissue factor VIIa complex activates factors IX and X that bind to activated platelets as well as other lipid surfaces and in concert with specific cofactors generate a macro-molecular prothrombinase complex (1,4). The subsequently formed thrombin proteolytically converts soluble fibrinogen into insoluble fibrin, activates the transglutaminase factor XIII leading to cross linking of the fibrin and activates platelets and endothelial cells. Thrombin also has a remarkable feed back am-

From the Department of Medicine, Weill Medical College of Cornell University, New York Presbyterian Hospital, 525 East 68th Street, BOX 130, New York, NY 10021.

Address for reprints: Department of Medicine, Weill Medical College of Cornell University, New York Presbyterian Hospital, 525 East 68th Street, BOX 130, New York, NY 10021.

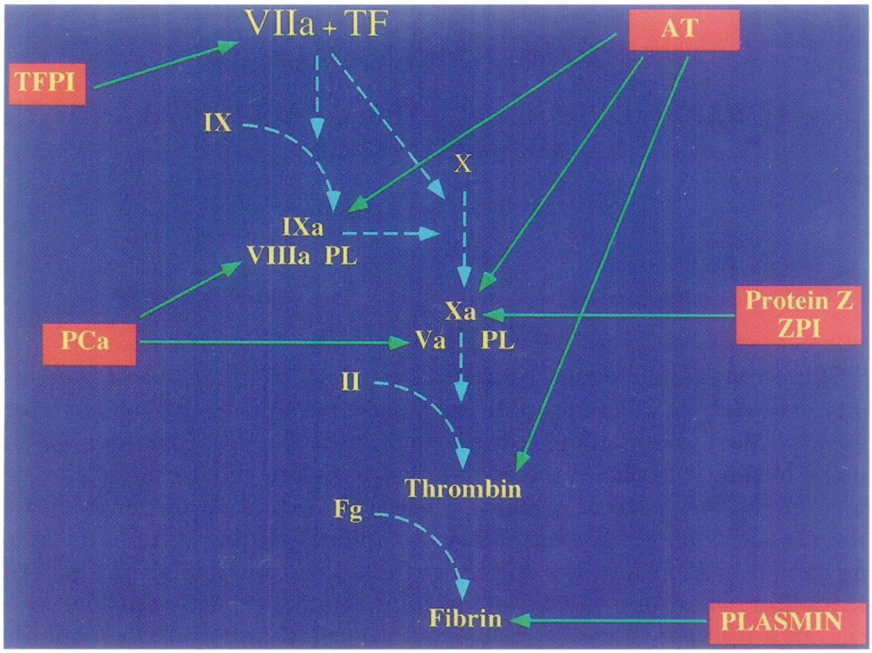

FIG. 1. Blood coagulation and anticoagulant inhibitory systems. Five separate anticoagulant control systems are indicated in red and the sites of action are shown by green arrows. Antithrombin (ATIII); Protein Z, Z pathway inhibitor (ZPI); plasmin; activated protein C (PCa); tissue factor pathway inhibitor (TFPI). The coagulation factor interactions are indicated by blue arrows originating with the formation of the VIIa tissue factor complex and culminating in the formation of fibrin.

plification effect by activating factors V, VIII, and XI (not shown). An alternative activating mechanism (the intrinsic pathway) involves factor XII, and high molecular weight kininogen that convert the zymogen factor XI to its active form which in turns activates factor IX (5). Feedback amplification loops also include factors VIIa, IXa, and Xa. It is obvious that the forward prothrombotic potential of the system is enormous and also potentially dangerous considering the potency of the key effector enzyme-thrombin.

It is therefore not surprising that thromboregulatory anticoagulant loops exist at each step of the cascade that inhibit activated enzymes or inactivate the associated cofactors. To date, five major anticoagulant inhibitory systems have been identified that control intravascular thrombin generation. (Table 1)

Antithrombin inhibits the free enzymes IXa, Xa and thrombin, and essentially limits the clotting process to the actual site of injury, preventing circulation of the activated zymogens and runaway cascade

TABLE 1
Anticoagulant Physiology

System	Targets
Antithrombin	IXa Xa Thrombin
Protein C (S)	Va VIIIa
TFPI	VIIa-Tissue Factor
Protein Z	Xa
Plasmin	Fibrin

activation (6). Access of IXa and Xa in their respective cofactor lipid complexes to anti thrombin-heparin is sterically limited. The efficiency of antithrombin as a serine protease inhibitor is increased by 3 logs following modulation by heparin. In vivo, the heparans on the endothelial cell surface enhance antithrombin inhibitory activity.

The protein C anticoagulant system plays a major role in the modulation of intravascular coagulation by targeted inactivation of the two major non enzymatic factors VIIIa and factor Va (7). Protein C, a vitamin K dependent zymogen is activated on endothelial surfaces by thrombin bound to a specific receptor thrombomodulin. Thrombomodulin bound thrombin activates Protein C but no longer posses procoagulant activity and is unable to activate platelets or endothelial cells and cannot cleave fibrinogen or activate factor XIII (8). This unique alteration in enzyme specificity endows the enzyme with anticoagulant activity because activated Protein C in concert with Protein S, another Vitamin K dependent protein, proteolyses and inactivates VIIIa and Va (Fig 2). Activated Protein C also cleaves intact factor V. This modified factor V acts in association with Protein S as a cofactor in the inactivation of factor VIIIa. Thus similar to thrombin, factor V depending on its molecular configuration can act as a procoagulant or an anticoagulant (9). Factor V Leiden, the most common inherited thrombophilic disorder, is characterized by resistance to activated Protein C and is due to a point mutation in the gene for factor V that codes for one of the specific cleavage sites for activated Protein C (10). The mutant protein factor V (R506 Q) is fully active as a procoagulant but is defective as an anticoagulant cofactor. The Protein C system is a major regulator of blood fluidity particularly in the capillary bed where there is a high density of thrombomodulin receptors. Endothelial cell Protein C receptor (EPCR) a second receptor system in large vessels has recently been described (11). This receptor binds Protein C and activated Protein C and facilitates anticoagulant action by positioning the zymogen in the proximity to thrombomodulin bound thrombin in the macrovasculature, such as the aorta.

Protein S circulates in two molecular forms. One free (30%) and one

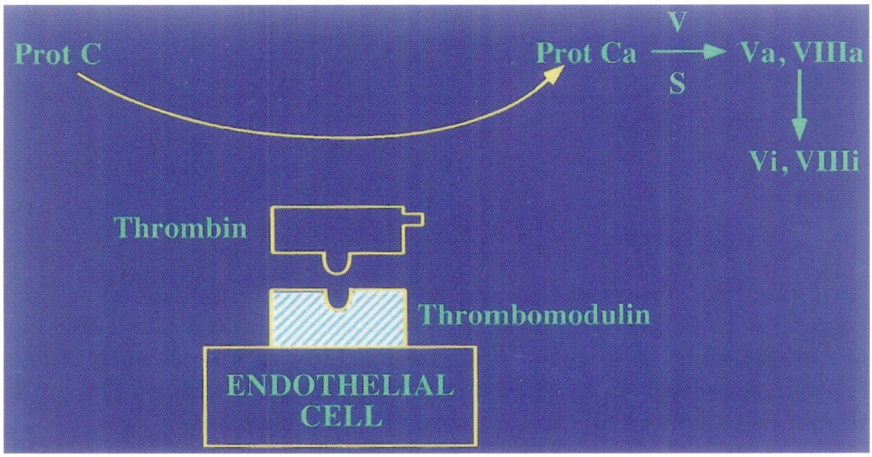

FIG. 2. Protein C anticoagulant system. Thrombomodulin on the endothelial surfaces binds thrombin altering the specificity of the enzyme. In the microvasculature, activated protein C is generated and a complex is formed on the cell surface with Protein S and factor V leading to the inactivation of Va and VIIIa (Vi and VIIIi).

bound to C4b binding protein (12). The free form is the functional co-factor for activated Protein C. Clinical conditions such as various inflammatory states leading to elevated levels of C4b binding protein shift the free/bound Protein S ratio and diminish the efficiency of the anticoagulant effect.

The tissue factor pathway inhibitor (TFPI) system down regulates the procoagulant activity of the tissue factor VIIa complex (13). The serine protease inhibitory activity is dependent on the generation of limited quantities of factor Xa and the formation of a multimolecular complex consisting of tissue factor, VIIa, Xa and TFPI. Endothelium constitutively expresses TFPI, which is also bound on smooth muscle cells, platelets, monocytes and tissue macrophages (14). To date, no inherited TFPI deficiency state has been described. TFPI gene disruption produces intrauterine lethality in mice. Presumably, deficiency states are also lethal in man. Heparin induces synthesis as well as secretion of TFPI from endothelial cells.

The Protein Z system is a newly described anticoagulant pathway that may play a physiologic role in coagulation regulation (15). Protein Z is a Vitamin K dependent protein that forms a complex with activated factor X on phospholipid surfaces. Inhibition of Xa is dependent on the presence of another plasma protein—Protein Z dependent protease inhibitor (ZPI). ZPI is a 72kDa serpin proteinase inhibitor that rapidly inhibits Xa in a purified system containing calcium, phospholipids and Protein Z. In mice Protein Z deficiency markedly increases

the thrombophilic phenotype of factor V Leiden (15). No abnormalities have yet been described in man.

The plasmin generating system may also be considered as part of an overall physiologically active thromboregulatory pathway. Plasmin digests fibrin and under pathologic circumstances may also proteolyze factors V, Von Willebrand factor and other coagulation factors. Abnormalities of the plasmin generating system including dysplasminogenemia, hypoplasmingenemia, impaired synthesis or release of tissue plasminogen activator and increased levels of plasminogen activator inhibitor have all been reported as rare causes of inherited thromboembolic disorders (16,17,18,19). Thrombin activatable fibrinolysis inhibitor (TAFI) is a procarboxypeptidase that markedly suppresses fibrinolysis (20). Conversion to the active carboxypeptidase is significantly increased by thrombomodulin bound thrombin. Increased levels are associated with thrombosis. The clinical genetics of the system have not yet been defined.

Endothelial Cell Thromboregulation

The thromboregulatory controls listed above and illustrated in Figure 1 take place primarily at the endothelial surface (Figure 3). The major constitutive function of normal endothelium is the maintenance of normal blood fluidity and perpetuation of the non-thrombogenic state. The different mechanisms as illustrated include the thrombomodulin Protein C system, the TFPI system, the plasmin generating system, and the antithrombin system. All of these physiologic pathways directly involve endothelial cell membrane activities. It is not yet known whether the Protein Z system involves the endothelial surface but it seems probable given the precedent of targeted protease inhibition at the site of vascular injury. Impaired regulation of these endothelial cell functions forms the basis of much of the pathophysiologic events associated with the development of primary hypercoagulable states.

Other constitutive endothelial activities contribute significantly to the thromboregulatory potential of the blood vessel wall. CD39, an ecto ADPase on the endothelium directly controls micro environmental ADP and modulates platelet accumulation and recruitment at sites of vascular injury (21). Nitric oxide generated by endothelial nitric oxide synthase stimulates quanylate cyclase which inhibits platelet reactivity and supports vascular relaxation. Similarly, endothelial cell prostacyclin is a potent inhibitor of platelet aggregation and promoter of vasodilation. Endoperoxides released from platelets are converted by

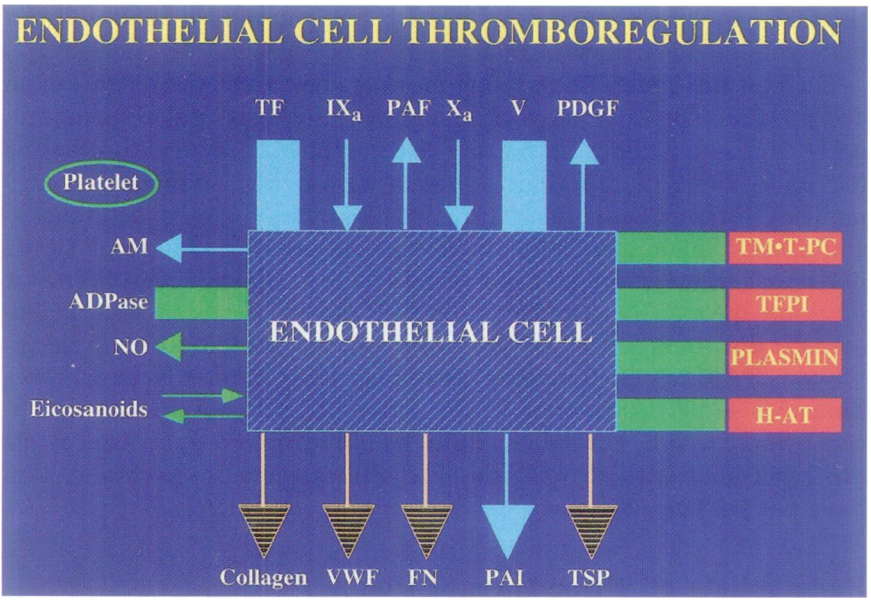

FIG. 3. Endothelial cell thromboregulation. Constitutive nonthrombogenic endothelial cell membrane functions (in green and red) include the thrombomodulin-thrombin-protein C system (TM-T-PC); tissue factor pathway inhibitor (TFPI); plasmin generating system (plasmin); the heparan antithrombin system (H-AT), ADPase; nitric oxide (NO) and the eicosanoid pathway. Nonconstitutive activated endothelial functions (blue) include adhesion molecules (AM); tissue factor (TF) IXa binding; platelet activating factor synthesis and release (PAF); factor V synthesis and assembly; platelet derived growth factor release (PDGF); and plasminogen activator inhibitor-1 release (PAI-1). Abluminal matrix deposition of thrombospondin (TSP), fibronectin (FN), von Willebrand factor (VWF) and collagen are shown by the pink arrows.

endothelial PGI_2 synthase to prostacyclin and released locally directly inhibiting platelet adhesion and aggregation (22). This "trans cellular" communication between endothelial cells and platelets is another mechanism that contributes to the antithrombotic control repertoire.

Systems Errors

Due to the complexity of the large number of overlapping systems involved, it is not surprising that a relatively large number of conditions can lead to the clinical phenotype of hypercoagulability. The basic abnormalities associated with these various clinical disorders are listed on Table 2. Inherited defects as well acquired abnormalities have been described as affecting the antithrombin, Protein C (S) and plasmin generating systems. No inherited defects have yet been described

TABLE 2
Hypercoagulability—System Errors

Antithrombin
Protein C (S)
Plasmin
TFPI
Protein Z (not yet)
Zymogen Kinetics (Polymorphisms)
Endothelial Regulation

involving the TFPI and Protein Z systems. TFPI activity increases in patients with poorly controlled Type 1 diabetes, particularly in those with microangiopathy. Enhanced TFPI expression has been observed in atherosclerotic vessels (23). The clinical spectrum of abnormalities associated with the Protein Z system remains to be clarified.

There is growing evidence that alterations in the kinetics of conversion of various coagulation factor zymogens to activated forms may contribute to the prothrombotic phenotype. Prothrombin 20210A is a relatively common polymorphism involving a substitution in the 3′ untranslated region of the prothrombin gene at nucleotide 20210, G to A (24). The increased risk of thrombosis associated with this polymorphism may reflect the higher absolute concentration of the zymogen in these patients. Similarly, elevated levels of factor XI, factor VIII, factor VII and fibrinogen have been associated with increased thrombosis (25,26,27,28). Recent studies suggest that certain polymorphisms of the factor VII gene may in fact lead to decreased levels of the zymogen, decreasing the risk of myocardial infarction in patients with coronary artery disease (29). This protective effect of lowered factor VII is also supported by clinical studies using low intensity warfarin in the primary prevention of coronary disease. Warfarin administration targeting an INR of 1.4 or greater reduced the risk of coronary event by 47% (30).

It is probable that a significant number of patients' altered coagulation factor levels reflect genetic polymorphisms that influence rates of transcription and translation. Some of the coagulation factors such as fibrinogen clearly respond to inflammatory cytokines and behave as acute phase reactants. Polymorphisms in the fibrinogen B chain may significantly influence the IL-6 response element controlling the acute phase response (31).

Control of endothelial cell function is a major factor in determining anticoagulant-coagulant balance. Endothelial cell activation downregulates the constitutive anticoagulant surface functions with conversion to a non constitutive proinflammatory thrombogenic phenotype.

This phenotype is associated with the surface expression of tissue factor, assembly of thrombin generating complexes, adhesion molecule and platelet activating factor expression and plasminogen activator inhibitor-1 secretion (32). In general, failure of the physiologically active endothelial steady state functions underlie most of the primary (inherited) hypercoagulable states while endothelial activation and conversion to a vascular thrombogenic phenotype forms the basis of the secondary (acquired) hypercoagulable states.

Some of these processes will overlap. Thus the thrombophilic state associated with the inherited MTHFR (methylene tetrahydrofolate reductase) polymorphism is associated with an elevated homocysteine level (33). Hyperhomocysteinemia has a direct down regulating effect on the constitutive anticoagulant functions of endothelium including decreased NO synthesis, inhibition of thrombomodulin activity and impaired tissue plasminogen activator binding with resultant diminished surface plasmin generation. Homocysteine also induces an active endothelial phenotype with associated increased tissue factor and plasminogen activator inhibitor-1 expression (34,35,36).

Primary Hypercoagulable States

These genetically determined disorders have been primarily associated with venous thrombosis (Table 3). Antithrombin, Protein C and Protein S hereditary deficiencies are found in approximately 5% of a random population of patients presenting with venous thromboembolism. The likelihood of detecting an abnormality is significantly increased in patients who present with specific clinical backgrounds. These include a strong family history, and thrombosis occurring at an early age or at an unusual site.

Factor V Leiden and Prothrombin 20210A are more prevalent in the general population and may be found in significant numbers of patients who present with an apparent idiopathic thrombotic event (37). Heterozygous prevalence for factor V Leiden in some Caucasian pop-

TABLE 3
Primary Hypercoagulability

Antithrombin Deficiency
Protein C Deficiency
Protein S Deficiency
Factor V Leiden
Prothrombin G20210A
MTHFR
Elevated VII VIII XI

ulations ranges up to 5–6% and is the most common genetic risk factor for venous thrombosis. The mutation is exceedingly rare in African Blacks and Asian populations. The mutation appears to have originated approximately 30,000 years ago and may convey an evolutionary advantage of the prothrombotic phenotype in peripartum hemorrhage. The prothrombin gene mutation in some populations may range up to 2% in prevalence and is the second most common genetic risk factor for venous thrombosis (38). Hyperhomocysteinemia (>18 μmol/L) has been found in 5% of the Dutch population and carries a 2–3 fold increased risk of thrombosis (39). The MTHFR enzyme constitutes one of the major enzymatic pathways for removal of homocysteine.

A number of other relatively less frequent genetic abnormalities have been reported to predispose to thrombotic disorders (Table 4). Elevated fibrinogen has been recognized as a significant risk factor for vascular occlusive events including myocardial infarction and stroke. β chain polymorphisms have been reported both with elevated fibrinogen levels as well as with increased atherogenesis. The associated thrombosis risk in this genotype is particularly strong in smokers. Polymorphisms on various platelet membrane glycoproteins including PL^{A2} on the β_3 integrin may be associated with enhanced risks for arterial thrombotic events due to altered adhesion, spreading and actin cytoskeletal rearrangement (40). Plasminogen activator inhibitor-1 (PAI-1) is a member of the serpin family and is a major modulator of tPA activity. Polymorphisms appear to influence gene transcriptional responses to Il-1 and predispose individuals to acute coronary syndromes (31). The PAI-1 genotype influences the course of atherothrombotic abnormalities. Because of the crucial role of thrombomodulin in the Protein C system, it is not surprising that genetic polymorphisms in this gene caused by altering expression as well as function of the receptor, are now being reported as potential risk factors in thrombotic disease. It is obvious that new genetic information in the coming years will greatly increase our knowledge of the relative importance of genetic risk factors for occlusive thrombotic disease.

TABLE 4
Primary Hypercoagulability—Rare

Fibrinogen
Platelet Glycoprotein Polymorphism
PAI-1 Polymorphism
Thrombomodulin Polymorphisms
Plasminogen

Secondary Hypercoagulable States (Table 5)

A large number of heterogeneous clinical conditions may predispose patients to thromboembolic events. Many of these disorders reflect cytokine mediated endothelial activation with conversion of the vascular wall from a constitutive nonthrombogenic surface to an active proinflammatory thrombogenic phenotype (32) (Figure 3).

In some of these conditions multiple effects occur including down regulation of normal endothelial steady state anticoagulant functions. Thus, acquired homocysteinemia 2° to folate and or B_{12} deficiency, directly activates the endothelium and also down regulates plasmin membrane assembly and Protein C activation. Similarly, multiple endothelial effects have been reported with antiphospholipid antibodies including endothelial cell activation with increased expression of leukocyte adhesion molecules ICAM, VCAM and E-selectin and inhibition of the Protein C system (35,41). In some of the conditions, endothelial cell dysfunction is coupled with concomitant platelet activation as is seen in heparin induced thrombotic states.

Cancer and concomitant hypercoagulability has been recognized for over a century. Idiopathic deep venous thrombosis without an identifiable risk factor including genetic predisposition, surgery, trauma, or pregnancy carries a significant risk for developing clinically overt cancer with an incidence of approximately 10% (42). The pathophysiologic processes associated with this relationship are not fully understood but include increased expression of macrophage and tumor cell tissue factor, down regulation of plasmin generation and impaired Protein C action.

Hepatic or portal-mesenteric venous thrombosis may be associated with primary myeloproliferative disorders including polycythemia vera, essential thromboythemia and myeloid metaplasia (43). Abnormal platelet function in these diseases probably contributes to the thrombotic process. The anatomic site predisposed in these specific disorders for the thrombotic event remains something of a mystery and

TABLE 5
Secondary Hypercoagulable—States

Cancer
Pregnancy
Nephrotic Syndrome
Myeloproliferative Disorders
Phospholipid Antibody Syndrome
Drugs
Postoperative State
Homocysteinemia

raises the question as to the geographic factors that determine a focal thrombotic lesion. Local organ pathology, such as a large spleen or a compressing invasive tumor obviously may contribute to stasis, viscosity alterations and/or focal endothelial dysfunction. Intrinsic endothelial cell heterogeneity with specific vascular bed signaling networks also appears to contribute significantly to the different clinical spectra with acquired as well as, inherited thrombotic phenotypes (Table 6) (44). Thus, individual clinical hypercoagulable syndromes preferentially involve different anatomic sites because of the specialized characteristics of the endothelial cell in a given organ environment. Protein C deficiency, Factor V Leiden, and Prothrombin G20210A are preferentially associated with the Budd-Chiari syndrome and portal vein thrombosis but not protein S or antithrombin deficiency (45). The orchestration of multiple factors including inherited genetic predispositions together with the specific environmental setting, i.e., a unique chemokine-cytokine profile in a given organ, will determine the clinical thrombotic phenotype. It is clear from these considerations that much of thrombotic disease results from multiple factors acting in concert at a specific point in time and place. Thus, an individual with an inherited thrombogenic predisposition such as heterozygous antithrombin deficiency, may develop a thromboembolic event during pregnancy or following trauma or immobilization following surgery. Multigenic effects markedly enhance the thrombogenic phenotype. The risk of thrombosis in patients who have combined Protein C deficiency and Factor V Leiden is 4–5 greater than in individuals who inherit a single genetic abnormality (37). The gene-gene interaction is particularly severe in the rare homozygous state involving Protein C or Factor V Leiden. Thrombotic risk with homozygous V Leiden deficiency is approximately 80 fold greater than the heterozygous state (46) (Table 7). The multicausal amplification of several factors acting synergistically,

TABLE 6
Thrombosis Geography

Disorder	Site
Protein C (S) Deficiency	Deep Veins
Antithrombin Deficiency	Deep Veins
V Leiden	Deep Veins
Prothrombin G20210A	Veins, Legs & Brain
PNH	Portal, Hepatic Veins
Myeloproliferative States	Portal, Hepatic Veins
Warfarin Skin Necrosis	Subq Microvasculature
TTP	Microvasculature—NOT Lung and Liver
Phospholipid Antibody Syndrome	Veins and Arteries

TABLE 7
Thrombosis—Relative Risk

Condition	Risk
Hyperhomocysteinemia	2.5
Oral Contraceptives	4.0
V Leiden +/−	4.0
Contraceptives and V Leiden	30.0
V Leiden −/−	80.0
Pro G20210A	2.5
Pro G20210A and Contraceptives	100.0*

* Cerebral Sinus Thrombosis.

is illustrated in women with heterozygous factor V Leiden deficiency who are on oral contraceptives. Here the relative rises from 4× to 30× (37). In certain rare thrombotic situations such as cerebral sinus thrombosis, the combination of Pro G 20210 A and oral contraceptives increased the risk 100× (47). It is fair to say that at the present time, most patients (60–70%) with a presumed "idiopathic" thromboembolic event after a complete evaluation will be shown to have a minimum of one underlying abnormality.

PREGNANCY

Pregnancy in the absence of currently identifiable predisposing factors is associated with an approximate 5–10× risk factor for venous thromboembolism (48,49). Pulmonary embolism is a leading cause of maternal death. Risk is markedly enhanced (30–50×) in the presence of heterozygous Protein C, Protein S or antithrombin deficiency (49). Factor V Leiden and Prothrombin G20210A heterozygous deficiencies carry lesser risks in pregnancy. It is important to note that these risks carry over into the post partum period and thus, if the patient requires therapy in the ante partum period, it should be extended for 6 weeks following delivery.

Maternal hypercoagulability also contributes significantly to obstetrical complications including preeclampsia, abruptio, fetal growth restriction and spontaneous abortion (50). High titre antiphospholipid antibodies have been associated with placental infarction and fetal loss (51). Inherited thrombophilic genetic predispositions also give rise to these syndromes. In a case-control study, the prevalence of genetic mutations in otherwise healthy women who had severe obstetrical complications, was greater than 50%. The major abnormalities included Factor V Leiden, MTHFR and Prothrombin G20210A. Sixty-five percent of the women with major complications had an inherited and/or acquired thrombophilic condition (50,52).

Screening and Management

Patients who present with recurrent, familial, or juvenile deep venous thrombosis or thrombosis in an unusual site (cerebral or mesenteric vein) should be screened for an underlying inherited abnormality including the current major players (Table 3). In persuasive clinical settings in the absence of clearly identifiable abnormalities, some of the rarer possibilities should be considered (Table 4). All of these patients should also be evaluated for homocysteine levels and antiphospholipid antibodies. Women who have had a serious obstetrical complication even in the absence of a recognized thromboembolic event should be evaluated for antiphospholipid antibodies, Protein C, Protein S and antithrombin deficiency as well as factor V Leiden, MHTFR polymorphism and Prothrombin G20210A.

Because thrombotic risks and mortality are low in untreated asymptomatic carriers, prophylactic anticoagulation should be restricted to specific situations known to be associated with increased risks such as surgery or prolonged immobilization. Patients should also receive counseling regarding avoidable risks such as smoking and the use of oral contraceptives. This is particularly true in carriers of antithrombin deficiency, Protein C deficiency and Protein S deficiency. In these latter conditions, estrogen replacement therapy probably should also be curtailed. Prophylactic anticoagulation in a pregnant woman without a previous thromboembolic event, is currently advised for antithrombin deficiency. Pregnant women with asymptomatic Protein C or Protein S deficiency should be followed carefully with periodic ultrasound imaging (49). Pregnant women with a previous history of a venous thromboembolic event and any predisposing inherited or acquired (phospholipid antibody) abnormalities, should receive low molecular weight heparin prophylaxis throughout pregnancy and 6 weeks into the post partum period.

Life long prophylaxis with oral anticoagulants is recommended for patients with inherited abnormalities who have had (1) multiple thromboembolic events (2) a single life threatening event such as a major pulmonary embolus (3) a single unprovoked major thromboembolic event in patients with antithrombin, Protein C or Protein S deficiency or in the setting of multiple separate gene abnormalities.

SUMMARY

Thromboregulatory physiology is essentially a function of the blood vessel wall. Constitutive endothelial cell activities maintain blood fluidity by down regulating the initiation as well as, the propagation of

blood coagulation. The major systems involved include: the Protein C, TFPI, plasmin generating and antithrombin pathways, all of which are focused on the cell membrane. Altered regulation of these endothelial functions forms the basis of the pathophysiologic events associated with the inherited primary hypercoagulable states. Secondary hypercoagulable syndromes occurring in various clinical states with conversion to a vascular thrombogenic phenotype reflect non constitutive activated endothelial cell functions with concomitant down regulation of the constitutive anticoagulant surface activity. So called idiopathic clinical thrombosis in most circumstances represents multi hit events in which specific genetic abnormalities or polymorphisms together with specific acquired alterations in geographically distinct endothelial cell beds culminate in a recognizable coagulation phenotype.

REFERENCES

1. Mann KG, Lorand L. Introduction: blood coagulation. Methods Enzymol 1993;222:1–10.
2. Furie B, Furie BC. Molecular and cellular biology of blood coagulation. N Engl J Med 1992;326:800–06.
3. Dahlbäck B. Blood coagulation. Lancet 2000;355:1627–1632.
4. Mann KG, van't Veer C, Cawthern K, Butenas S. The role of the tissue factor pathway in initiation of coagulation. Blood Coagul Fibrinolysis 1998;9:S3–7.
5. Gailani D, Broze GJ Jr. Factor XI activation in a revised model of blood coagulation. Science 1991;253:909–12.
6. Rosenberg RD. Coagulation—fibrinolytic mechanism and the action of heparin. Adv Exp Med Biol 1975;52:217–237.
7. Dahlback B. The protein C anticoagulant system: inherited defects as basis for venous thrombosis. Thromb Res 1995;77:1–43.
8. Esmon CT. The regulation of natural anticoagulant pathways. Science 1987;235:1348–1352.
9. Dahlback B. Procoagulant and anticoagulant properties of coagulation factor V: factor V Leiden (APC resistance) causes hypercoagulability by dual mechanisms. J Clin Lab Med 1999;133:415–22.
10. Thorelli ET, Kaufman RJ, Dahlback B. Cleavage of factor V at Arg 506 by activated protein C and the expression of anticoagulant activity of factor V. Blood 1999;93:2552–2558.
11. Fukudome K, Ye Xiaofev, Tsuneyoshi N, et al. Activation mechanism of anticoagulant protein C in large blood vessels involving the endothelial cell protein C receptor. J Exp Med 1998;187:1029–1035.
12. D'Angelo A, Vigano D'Angelo S, Esmon CT, Camp PC. Acquired deficiencies of protein S. Protein S activity during oral anticoagulation in liver disease and in disseminated intravascular coagulation. J Clin Invest 1988;81:1445–1454.
13. Broze GJ Jr. Tissue factor pathway inhibitor. Thromb Haemost 1995;74:90–93.
14. Bilsel AS, Onaran N, Moini H, Emerk K. Long-term effect of 17beta-estradiol and thrombin on tissue factor pathway inhibitor release from HUVEC. Thromb Res 2000 Jul 15;99(2):173–178.

15. Yia LF, Zhong-Fu H, Cui J, et al. Prothrombotic phenotype of protein Z deficiency. PNAS 2000;97:6734–6738.
16. Aoki N, Moroi M, Sakata Y, Yoshida N, Matsuda M. Abnormal plasminogen. A hereditary molecular abnormality found in a patient with recurrent thrombosis. J Clin Invest 1978;61:1186–1195.
17. Lottenberg R, Dolly FR, Kitchens CS. Recurrent thromboembolic disease and pulmonary hypertension associated with severe hypoplasminogenemia. Am J Hematol 1985;19:181–193.
18. Hamsten A, Wilman B, de Faire U, B Blombach B. Increased plasma levels of a rapid inhibitor of tissue plasminogen activator in young survivors of myocardial infarction. N Engl J Med 1985;313:1557–1563.
19. Mikkelsson J, Perola M, Wartiovaara U, Peltomev L, et al. Plasminogen activator inhibitor-1 4G/5G polymorphism, coronary thrombosis, and myocardial infarction in middle aged Finnish men who died suddenly. Thromb Haemost 2000;84:78–82.
20. Van Tilburg NH, Rozendaal FR, Bertina RM. Thrombin activatable fibrinolysis inhibitor and the risk for deep vein thrombosis. Blood 2000;95:2855–2859.
21. Marcus AJ, Broekman MJ, Drosopoulos JH, et al. The endothelial cell ecto-ADPase responsible for inhibition of platelet function is CD39. J Clin Invest 1997;99:1351–1360.
22. Marcus AJ, Weksler BB, Jaffe EA. Enzymatic conversion of prostaglandin endoperoxide H2 and arachidonic acid to prostacyclin by cultured endothelial cells. J Biol Chem 1978;253:7138–7143.
23. Crawley J, Lupu F, Westmackett AD, Severs NJ, Kakkar VV, Lupu C. Expression, localization, and activity of tissue factor pathway inhibitor in normal and atherosclerotic human vessels. Arteriosler Thromb Vasc Biol 2000;20:1362–1373.
24. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3′-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood 1996;88:3698–703.
25. Meijers SCM, Teklenburg WLH, Bouma B, Bertina RM, Rosendahl FR. High levels of coagulation factor XI as a risk factor for venous thrombosis. N Engl J Med 2000;342:696–701.
26. Kyrle PA, Mina E, Hirsch IM, et al. High plasma levels of Factor VIII and the risk of recurrent venous thromboembolism. N Engl J Med 2000;343:457–462.
27. Meade TW, Mellows S, Brozovic M, et al. Haemostatic function and ischemic heart disease: principal results of the Northwick Park Heart Study. Lancet 1986;2:533–537.
28. Ernst E, Resch KL. Fibrinogen as a cardiovascular risk factor: a meta analysis and review of the literature. Ann Int Med 1993;118:956–963.
29. Girelle D, Russ C, Ferraresi P, et al. Polymorphisms in the factor VII gene and the risk of myocardial infarction in patients with coronary artery disease. N Engl J Med 2000;343:774–780.
30. MacCallum PK, Brenna PJ, Meade TW. Minimum effective intensity of oral anticoagulant therapy in primary prevention of coronary heart disease. Arch Int Med 2000;160:2462–2468.
31. Lane DA, Grant PJ. Role of hemostatic gene polymorphisms in venous and arterial thrombotic disease. Blood 2000;95:1517–1532.
32. Silverstein RL. The Vascular Endothelium in Inflammation: Basic Principles and Clinical Correlates. Third Edition. Editors F. Gallin, Snyderman. Lippincott Williams and Wilkins. Phil. 1999;Chapter 15:207–223.
33. Frosst P, Bloom HJ, Milo R, et al. A candidate genetic risk factor for vascular

disease: a common mutation in methylene tetrahydrofolate reductase. Nat Genet 1995;10:111–113.
34. Midorikama S, Savada H, Hashimoto S, Watanabe T. Enhancement of homocysteine of plasminogen activator inhibitor-1 gene expression and secretion from vascular endothelial and smooth muscle cells. Biochem Biophys Res Common 2000;272:182–185.
35. Pruefer D, Scalia R, Lefer M. Homocysteine provokes leukocyte-endothelium interaction by downregulation of nitric oxide. Gen Pharmacol 1999;33:487–498.
36. Hajjar KA, Jacovina AT. Modulation of annexin II by homocysteine: implications for atherothrombosis. J Invest Med 1998;46:364–369.
37. Rosendaal FR. Venous thrombosis: a multicausal disease. Lancet 1999;353:1167–1173.
38. Rosendaal FR, Doggen CJM, Zivelin A, et al. Geographic distribution of the 20210A prothrombin variant. Thromb Haemost 1998;79:706–708.
39. Den Heigger M, Rosendaal FR, Blom HJ, Gerrit WBJ, Bos GMJ. Hyper homocysteinemia and venous thrombosis: a meta analysis. Thromb Haemost 1998;80:874–877.
40. Byzova T, Plow EF. The Pl^{A2} allele and cardiovascular disease: the pro^{33} and con. J Clin Invest 2000;105:697–698.
41. Dudman NP, Temple SF, Guo XW, Fu W, Perry MA. Homocysteine enhances neotrophil—endothelial interactions in both cultured human cells and rats in vivo. Circ Res 1999;84:409–416.
42. Prandoni P, Lenising AWA, Buller HR, Cogo A, Prins MH, Cattelan AM. Deep vein thrombosis and the incidence of subsequent symptomatic cancer. N Engl J Med 1992;327:1128–1133.
43. Valla D, Casadevall N, Lacombe C, et al. Primary myeloproliferative disorder and hepatic vein thrombosis. Ann Inter Med 1985;103:329–334.
44. Rosenberg RD, Aird WC. Vascular-bed-specific hemostasis and hypercoagulable states. N Engl J Med 1999;340:1555–1564.
45. Janssen HLA, Meinardi JR, Viegaar FP, et al. Factor V Leiden mutation, prothrombin gene mutation, and deficiencies in coagulation inhibitors associated with Budd-Chiari syndrome and portal vein thrombosis: results of a case control study. Blood 2000;96:2364–2368.
46. Rosendaal FR, Koster T, Vaudenbroucke JP, Reitsma PH. High risk of thrombosis in patients homozygous for factor V Leiden. Blood 1995;85:1504–1508.
47. Martinelli I, Sacchi E, Landi G, Taioli E, Duca F, Manucci PM. High risk of cerebral vein thrombosis in carriers of the prothrombin gene mutation and in users of oral contraceptives. N Engl J Med 1998;338:1793–1797.
48. Treffers PE, Huidekoper BL, Weenink GH, Kloosterman GJ. Epidemeological observations of thromboembolic disease during pregnancy and in the puerperium in 56,022 women. Int J Gynaecol Obstet 1983;21:327–331.
49. Kearon C, Crowther M, Hirsh J. Management of patients with hereditary hypercoagulable disorders. Annu Rev Med 2000;57:169–185.
50. Kupferminc MJ, Eldor A, Steinman N, et al. Increased frequency of genetic thrombophilia in women with complications of pregnancy. N Eng J Med 1999;340:9–13.
51. Greaves M. Antiphospholipid antibodies and thrombosis. Lancet 1999;353:1348–1353.
52. Greer IA. Thrombosis in pregnancy: maternal and fetal tissues. Lancet 1999;353:1258–1265.

DISCUSSION

Douglas, Princeton: I was going to ask you one question, and that was; everybody tends to study one cell or one system and many of these proteins have multiple activities. I wondered if there is anything in the whole blood clotting system—what's known about activities on brain cells and other kinds of cells in the body?

Nachman, New York: We are just beginning to scratch this biological surface. For instance, tissue factor can initiate cell signals and alter cell phenotype. Some of the coagulant factors probably initiate physiologic responses independent of thrombin generation. How important this is remains to be determined.

Copeland, LaGrange: Really a spectacular, lucid presentation—thank you. Two small points. One you mentioned at the beginning that hypercoagulability was a burden to the western world. Is there any reason to think that the non-western world is any different?

Nachman: The genetic burden of inherited thrombophilic states differs in different populations so that founder effects will be predominant in one geographic area and minimal in another region.

Copeland: Would you comment on screening of patients that may have hypercoagulability who are already on anticoagulants in one form or another?

Nachman: Some of the specific protein factors can be easily measured with immunological assays or genetic screens and can thus be evaluated even in the presence of heparin. Some factor levels are influenced by coumadin. In general, I wait until the acute event is over and initiate a screening process later tailored to the specific clinical setting.

Kitchens, Gainesville: An excellent paper. Two things happened in 1980's related to hemophilia, and I am wondering even if they are coincidental. The explosion of information that we the clotter group made on thrombosis and the loss of 90% of our patients to HIV. Is that coincidental or not?

Nachman: Many of our patients with hemophilia in the early 80's were infected with HIV due to unrecognized contamination of the blood products. Certainly this devastating development acted as a catalyst in generating new knowledge regarding the molecular and cellular biology of the blood coagulation system.

Kitchens: I think we have something to do. We lost our population base and our attentions for the first time since fifties went from bleeding to thrombosis.

Nachman: Yes, but the fact of the matter is that at the same time the world of polymorphisms and the world that Victor McKusick created has helped broaden our whole understanding of biology and the ability to knock out each of these genes in mice gives you incredibly interesting phenotypes which you then can extrapolate back to man. So I think it's a lot of things. I think the explosion of genetics and the explosion of new technologies to manipulate genetics to simulate human disease in animals is probably the major insight that we have.

Sande, Salt Lake City: Ralph, we went through a phase about thirty years ago when we were anticoagulating patients with meningococcemia with heparin. This is no longer recommended. Given what you know today, how would you treat that patient?

Nachman: Activated protein C has shown to be effective in experimental models of gram negative sepsis and is moving into clinical use. A recent study has shown effectively in purpura fulminans associated meringicoccenia.

Sande: So is this available now?

Nachman: There have been studies launched, and I believe APC will be coming down the line in terms of human therapeutics.

Colwell, Charleston: There's a growing view among diabetologists that Type 2 diabetes may be a pro-thrombotic or hypercoagulable state. It is based primarily on

platelet activation endothelial abnormalities and in high plasma and platelet plasminogen activators one (PAI-1) levels, and of course the clinical endpoints of thrombotic arterial disease. Would you comment on that concept?

Nachman: I think it is a reasonable premise. Diabetes directly influences endothelial cell function via advanced glycosylation end products.

Santen, Charlottesville: Ralph, I think the evidence is accumulating now that hormone replacement therapy and the SERMs, tamoxifen and relaxofene are associated with an increased incidence of DVT and pulmonary emboli. How many of these individuals do you think have a second hit (i.e., second underlying genetic defect) and should we perhaps be thinking about screening individuals that are going to go on hormone replacement therapy for predisposing factors and in particularly for some of the defects that you have outlined?

Nachman: Oral contraceptives increase the risk of a thrombotic event particularly in carriers of antithrombin deficiency, protein C deficiency or protein S deficiency. In young women with familial thrombotic histories, screening is clearly indicated before initiating oral contraceptions. I would also apply this rule to older women before initiating hormonal replacement therapy. In a completely asymptomatic state with a negative family history screening is probably not indicated.

Sacher, Washington: The 20210 mutation is so prevalent in many patients with thrombophilia. Most of the cases that we do PCR it's heterozygous. I've never seen a homozygote. Do you think homozygosity is incompatible with life?

Nachman: I believe that the homozygous state is not lethal.

Palmer, Ardmore: Ralph two quick questions. If you put all of these small defects or these relatively rare defects together, what proportion of the population do you believe is at risk of increased coagulability? And the second question is, what implications do think it has that there's now such wide-spread use of the COX-2 selective NSAID that inhibit prostacycline without at the same time affecting thromboxane?

Nachman: Let me answer your last question first. I think we are in for surprises in the next couple of years about the adverse effects on human biology of COX-2 inhibition. Particularly in patients who are also taking aspirin. Nature didn't make man or woman to be free of the COX enzymes. So I think that there maybe adverse circumstances that we will see in patients who have both hits.

Palmer: If you put all these smaller disorder together, what proportion of the population is at risk for hypercoagulability?

Nachman: We don't know the precise number but I would estimate that at present well over 60% of hypercoagulable clinical conditions are associated with underlying inherited predispositions.

Gotto, New York: It's really an excellent lecture Ralph, and you'll probably be asking me for a raise next year, you deserve it. I wanted to ask you about your view about the most promising targets in coagulation in the precoronary syndrome phase. That is going from the stable plaque to the unstable plaque for the patient with the acute coronary syndrome there probably is not enough endothelial cells there to have that as a target. We know IIBIIIA inhibitors are effective in the acute coronary syndrome, A-Z trial is testing also the relative efficacy of unfractionated heparin versus low molecular weight heparin. But if you move back to the stable plaque, is there enough endothelium intact at that point to make endothelium a primary target? What do you think would be the best target?

Nachman: There is no clear cut answer yet available. Recent studies suggest to me that impaired thrombin generation such as is seen in patients on low levels of coumadin with an INR in the 1.4 or higher range may offer primary protection in patients with stable disease. The trade off of hemorrhagic complications will be the crucial determi-

nant of clinical usefulness. It is of interest that a Factor VII polymorphism with lower levels to the 75% range seems to offer protection from thrombosis.

Abboud, Iowa City: I am intrigued by the failure of the thrombomodulin system to be expressed specifically in the cerebral endothelial tissue. Any insight into the cause of this selectivity? Are these selective properties preserved in vitro in endothelial cultures from various vascular beds?

Nachman: In large vessels like the aorta, thrombomodulin is very ineffective, because its presence is scattered over large distances compared to some capillary beds in which it is densely represented. And there is another receptor in the large vessel called the endothelial protein C receptor EPCR, that augments the effect of thrombomodulin by several logs.

Abboud: As you become much more sophisticated in the specificity of coagulant molecules, how far are we from specifically targeted anticoagulant molecular treatment in different patients? Instead of giving just coumadin across the board, as a general anticoagulant to everyone.

Nachman: This is a rapidly moving field with new therapeutic agents coming down the pike. These include specific factor Xa inhibitors, oral antithrombins, specific tissue factor inhibitors, and a new pentasaccharide low molecular weight heparin.

Weissler, Rochester: On the thrombotic propensity of the fibrillating left atrium, what do you think is the underlying mechanism for the thrombi and emboli which result?

Nachman: Beside the obvious evidence of stasis?

Weissler: How do the mechanical factors of stasis lead to thrombosis?

Nachman: I guess what you are saying, what are the specific changes in the fluid nature of the blood and that environment, and is there anything different on the endocardium of the left-atrium? That's a good question. We do know that hydrodynamic flow alters specific gene expression in the endothelium leading to a procoagulant effect.

Boyer, Hamden: Ralph, are there differences in the rate of reabsorption of clots depending on the genetic background?

Nachman: A number of inherited thrombophilic conditions are associated with abnormalities of fibrinolytic factors including plasminogen, t-pa and PAI-1. Presumably the clinical phenotype reflects defects in the rate of fibrin dissolution.

Boyer: One last question, why does PNH predispose to hepatic vein thrombosis? What is it about PNH that leads to clotting?

Nachman: This remains a clinical mystery. There are probably some unique thrombophilic characteristics of the hepatic vein endothelial cell bed.