Thrombophilia and Hypercoagulability

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Key Words

Thrombophilia · Hypercoagulability · Antiphospholipid syndrome

Abstract

This is a review of less well-known aspects of thrombophilia and hypercoagulability as they relate to thrombosis. Thrombosis is an abnormal fibrin clot that develops in circulating blood with clinical symptoms of one or more arterial and/or venous obstructions exclusively identified by imaging techniques. The terms thrombophilia and hypercoagulability are often used indiscriminately when they are in fact separate entities. Thrombophilia is an inherited or acquired clinical phenotype manifesting in selected individuals as a greater risk to develop recurrent thrombosis at a younger age than the general population, with considerable differences in the magnitude of risks among individuals in the same family with the same thrombophilic gene defect. Hypercoagulability is a laboratory phenotype whereby in vivo activation of clotting, fibrinolysis, endothelial cells and platelets is identified in vitro by specialized clotting techniques and by specific antibodies directed at biomarkers of clotting activation and damaged vasculature. Hypercoagulability may be provoked by drugs to treat bleeding in hemophilia, by sepsis, inflammation, surgery, blood stasis, atherosclerosis, and it manifests selectively in inherited and acquired thrombophilia. A chronology of the discovery of acquired and inherited thrombophilia puts in perspective the data analyzed in

two representative large family studies that address whether venous and arterial thrombosis are a necessary outcome in thrombophilia, and the question, whether patients with inherited antithrombin, protein C and protein S deficiencies need to be treated after a first episode of thrombosis. The liberal use of case vignettes emphasizes a close relationship and the distinction between thrombosis, thrombophilia and hypercoagulability.

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Introduction

Interest in thrombophilia/hypercoagulable state and hypercoagulability is so widespread that a search in Medline's electronic database for articles published in the past 8 years (2000–2008) yielded 30% more entries for thrombophilia (19,029) than for coronary artery thrombosis (13,558), the number one cause of mortality in the industrialized world. Moreover, clinical manifestations of congenital and acquired thrombophilias, evidence-based indications for arterial and venous thrombophilia screening and the choice and duration of anticoagulation are reviewed in 3,376 publications from the year 2000 to the present, and in the invited article by Dahlback [1] celebrating the 50th anniversary of the American Society of Hematology. The intent of this review is to: (a) present a chronology of the discovery of acquired and inherited thrombophilias; (b) provide evidence that individuals

Fig. 1. Antigenic targets of autoimmune antiphospholipid antibodies are viral or bacterial proteins with sequence similarity (molecular mimicry) to the phospholipids shown.

Fig. 2. Autoimmune antiphospholipid antibodies are induced by binding of β_2 -glycoprotein 1 to phospholipid viral or bacterial proteins with sequence similarity (molecular mimicry) to the phospholipid binding region of β_2 -glycoprotein 1/lysine rich phospholipid binding region.

with thrombophilia do not necessarily develop thrombosis during their lifetime; (c) elucidate the question whether patients with inherited antithrombin, protein C and protein S deficiencies need to be treated after the first episode of thrombosis; (d) define the hemostatic state and describe biomarkers of clotting, vascular damage and inflammation; (e) elucidate the hypercoagulability phenotype; (f) by the liberal use of case vignettes emphasize a close relationship, as well as the distinction between thrombophilia, hypercoagulability and thrombosis.

Chronology of the Discovery of Inherited and Acquired Thrombophilia

Antiphospholipid Syndrome

The antiphospholipid syndrome (APS) is an acquired thrombophilia and the first recognition of any acquired or inherited thrombophilia tied to complex interactions that result in venous and arterial thrombosis, obstetric complications and thrombocytopenia. The evolving history of APS began in 1963 with the pioneer discovery of the association of the lupus anticoagulant with thrombosis by E. Walter Bowie of the Mayo Clinic and his colleagues [2, 3]. The lupus anticoagulant, previously considered an inhibitor of clotting reactions linked to hemorrhagic disorders, was subsequently identified by the anticardiolipin test to belong to the family of antiphospholipid antibodies [4]. The association of antiphospholipid antibodies with thrombosis and obstetric events de-

fined the APS, and work done in the 1990s made it clear that the true antigenic targets of antiphospholipid antibodies are not the phospholipids but plasma proteins bound to an anionic surface. Depicted in figure 1 and 2 is an association of lupus anticoagulant antibody belonging to a class of antibodies that prolong clotting times in phospholipid-dependent clotting tests, with coagulation protein antigens and the antigen β_2 -glycoprotein 1 [5]. It is now confirmed that β_2 -glycoprotein 1 is the most common and best-characterized antigenic target of autoimmune antiphospholipid antibodies. β_2 -Glycoprotein 1, also known as apolipoprotein H, is an anticoagulant plasma protein belonging to the complement control protein family, and it is present in plasma at a concentration of approximately 150–300 $\mu\text{g/ml}$. β_2 -Glycoprotein 1 anticoagulant action is by interaction with anionic phospholipids via a lysine-rich region in the extra 20 amino acid C-terminal loop of the fifth domain that prevents thrombin amplification by the prothrombinase activity and prevents ADP-induced platelet aggregation [6]. Gharavi et al. [7] researched in mice and rabbits the mechanism by which autoimmune antiphospholipid antibodies develop in APS, and from results that were confirmed by others, they hypothesize, as depicted in figures 1 and 2, that autoimmune antiphospholipid antibody production in patients is induced by binding of β_2 -glycoprotein 1 to phospholipid viral or bacterial proteins. The sequence similarity (molecular mimicry) of these proteins to the phospholipid binding region of β_2 -glycoprotein 1 breaks the tolerance to self β_2 -glycoprotein 1 and induces anti-

bodies against the β_2 -glycoprotein 1/lysine-rich phospholipid binding region [7]. Autoimmune antiphospholipid antibodies act by either one or by all of the following mechanisms: (1) activation of complement [8], (2) induction of tissue factor expression and cell adhesion receptors on endothelial cells, and (3) promoting platelet aggregation [9]. A full account of the formalization of clinical classification criteria in APS is comprehensively presented by Harris and Pierangeli [10] in a recent publication. Three main differences between the original Sapporo classification and the revised Sydney criteria for APS are cited by Lockshin et al. [11] as (a) the exclusion of men aged 50 and over and women 65 and older because of competing causes for thromboembolic disease in older age groups, (b) the interval between the required confirmatory tests for APS is increased from 6 to 12 weeks, and (c) the addition of clinical and laboratory criteria that will qualify 6–25% of patients identified by thromboembolic disease to be diagnosed with APS. Clinically, APS is implicated in arterial and venous thrombosis and recurrent fetal loss and screening is required for pregnant women with a previous thrombosis or a positive history for APS [12]. Microthrombotic/microangiopathic manifestation of APS (Asherson's syndrome) is an uncommon but devastating manifestation of long-standing APS that further complicates the clinical picture by its close resemblance to thrombotic thrombocytopenic purpura, purpura fulminans and hemolytic uremic syndrome [13].

Antithrombin Anticoagulant Pathway

The report by Egeberg [14] in 1965 of an antithrombin type I autosomal dominant deficiency in a Norwegian family was the first recognition of a genotypic characteristic to predispose individuals to a heightened tendency to develop venous or arterial thrombosis, with thromboembolic events occurring relatively early in life [15]. The isolation and characterization of antithrombin from human plasma by Rosenberg and Damus [16] came much later in 1973.

Protein C Anticoagulant Pathway

In 1976 Stenflo [17] published the discovery of bovine protein C, a vitamin K-dependent factor and zymogen of a serine proteolytic enzyme. This was followed by several reports on the isolation, characterization, and mechanism of human protein C activation by α -thrombin and by proteases derived from the venom of southern copperhead [18–22]. The anticoagulant effect of activated protein C (APC) on factors V and VIII also was recognized at that time [23–27]. The first biochemical cause to be

identified by a persistence in blood of both activated factors V and VIII was a deficiency of protein C associated with thrombotic disease reported by Griffith et al. [28] in 1981, and a few years later, in 1984 Schwarz et al. [29] and Comp et al. [30] concomitantly reported familial protein S deficiencies associated with recurrent thrombosis. The role of protein S in the regulation of blood coagulation was reported by Walker and Fay [31] in 1993 and in 1985, the protein C gene was sequenced permitting the identification of DNA deleterious mutations [32]. Hassouna [33] recognized in 1991 a second biochemical cause, resistance to inactivation of both activated factors V and VIII by APC associated with thrombosis. After adding Protac®, a purified fraction of the venom of *Agkistrodon contortrix contortrix* to activate plasma protein C of a 33-year-old patient with recurrent thrombosis since age 21, both factor Va and factor VIIIa resisted inactivation [34, 35]. It was confirmed [Joseph P. Miletich, personal communication] that the protein C immunoreactive levels and the amount of protein C purified from the patient's plasma were 120% of normal, and genetic testing in 1999 identified a homozygous state for the factor V Leiden mutation reported in 1994 by Bertina et al. [36]. In 1993, by an innovative approach, expected to bypass protein C activation, Dahlbäck et al. [37] added APC to plasma from a patient with thrombosis and discovered that both factors Va and VIIIa resisted inactivation, which they named APC resistance. APC resistance is a laboratory clotting value measured in an assay wherein standardized amounts of APC added to plasma have no influence on plasma levels of protein C. APC resistance also became the clinical name applied to a familial thrombotic disorder, and the first notable exception of a clinical entity ascribed to a laboratory clotting value [37]. The genetic basis for most of the hereditary APC resistance cases (about 90%) was identified by Bertina et al. [36] in 1994 as a point mutation in the gene for coagulation factor V (fV R506Q). A prospective cohort study determined an annual incidence of 0.058% venous thromboembolism in asymptomatic carriers of the factor V Leiden mutation. The factor V Leiden mutation is considered a common polymorphism in the Caucasian race (40–50% carriers) and heterozygous carriers have not been reported with spontaneous unprovoked thrombosis. In 1996, the same group that identified the factor V Leiden mutation in the factor V gene reported a G-to-A substitution at nucleotide 20210 in the 3'-untranslated region of the prothrombin gene associated with increased prothrombin synthesis by the liver, elevated plasma prothrombin levels and an increased risk of venous thrombosis [38].

Thrombosis Is Not a Necessary Outcome in Thrombophilia and Patients with Hereditary Protein S, Protein C or Antithrombin Deficiency and Thrombosis Have a High Absolute Risk of Recurrence

Genetic polymorphisms and mutations as well as circumstantial changes in quantity or quality of factors that clot blood, or prevent blood clotting, constitute the majority of acquired and inherited thrombophilia disorders. The incidence of inherited thrombophilia is 24–37% in individuals with thrombosis compared to a 10% incidence in individuals without thrombophilia and with thrombosis [39–41]. Immediately after the discovery of the strong association of thrombosis and inherited thrombophilia, there was a sense that may persist to this day in some medical communities, that all individuals with inherited thrombophilia will inevitably develop thrombosis during their lifetime. Thirty years after the report by Egeberg [14] of inherited antithrombin deficiency causing thrombophilia, the World Health Organization/International Society of Thrombosis and Hemostasis in 1995 defined inherited thrombophilia as an unusual tendency toward thrombosis with early age of onset, strong family history, a degree of thrombosis severity out of proportion to a recognized stimulus and recurrent episodes of thrombosis [42]. The word ‘tendency’ judiciously corroborates the high prevalence of heterozygosity for protein C deficiency in individuals without thrombosis, a finding first reported by Miletich et al. [43] in the *New England Journal of Medicine* in 1987. Miletich et al. [43] conducted a study of protein C antigen levels in healthy blood donors and discovered a much higher than previously estimated number of heterozygote protein C carriers of 1/200–1/500.

The first retrospective cohort family study to provide evidence that individuals with thrombophilia do not necessarily develop thrombosis during their lifetime was conducted between 1980 and 1995 and published in 1998 [44]. The data gathered in this study also provides important information on differences in the thrombotic risk in individuals with deficiencies of the anticoagulant proteins with important implications for management strategies that are relevant to this day. Of 150 index patients with inherited deficiencies attending two Italian thrombosis centers in Milan and Rome, 723 first- and second-degree relatives were recruited in the study. Higher risks for thrombosis (table 1) were identified for subjects with antithrombin (risk ratio 8.1, 95% confidence interval, CI, 3.4–19.6), protein C (7.3, 95% CI 2.9–18.4) or protein S deficiency (8.5, 95% CI 3.5–20.8), and factor V Leiden

(2.2, 95% CI 1.1–4.7) than for individuals with normal coagulation. The risk of thrombosis for subjects with factor V Leiden was lower than that of those with all three other coagulation defects (0.3, 95% CI 0.1–1.6), even when arterial and superficial vein thromboses were excluded and the analysis was restricted to deep vein thrombosis (0.3, 95% CI 0.2–0.5). There was no association between coagulation defects and arterial thrombosis in that study. The most frequent venous thrombotic manifestation was deep vein thrombosis with or without pulmonary embolism (90% in antithrombin, 88% in protein C, 100% in protein S deficiency, and 57% in factor V Leiden), but a relatively mild manifestation such as superficial vein thrombosis was common in factor V Leiden (43%). The thrombosis risk is compounded by other heterozygous mutations or in association with a chronic disorder, with pregnancy, surgery or trauma [41]. In table 1 the heading ‘all thromboses’ includes all relatives with or without venous and arterial thrombosis; ‘venous thrombosis’ indicates superficial and deep venous thrombosis and excludes relatives with arterial thrombosis. ‘Venous thromboembolism’ includes relatives with deep venous thrombosis with exclusion of relatives with arterial thrombosis and superficial thrombosis. The incidence for thrombosis per 100 patient-years for each thrombophilic defect is indicated in the last column. The heading ‘yes’ is for relatives with thrombosis and ‘no’ is for relatives without thrombosis; of 85 family members diagnosed with antithrombin deficiency 55 never had a thrombosis, and 45 family members with protein C deficiency did not have thrombosis. This is the first large evidence-based study to indicate that the phenotype of heterozygous protein C deficiency, passed down as an autosomal or recessive trait, is similar to that of persons with inherited antithrombin deficiency and to prove that a fraction of individuals with inherited coagulation defects develop thrombosis while the majority live and die without experiencing thrombosis.

Not included in this study were individuals with the G-to-A substitution in the prothrombin gene present in 1–2% of healthy controls and in 18% of a referral population with a personal and family history of thrombosis. This mutation independently confers a 2.8-fold increased risk of venous thrombosis [38]. The reader is referred to electronic library searches beyond the scope of this review, for comprehensive data on the different risks of thrombosis and the risk of recurrence of thrombosis among patients with inherited antithrombin protein C and protein S deficiencies, and patients with the factor V Leiden and prothrombin G20210A polymorphisms.

Table 1. Relative risk and annual incidence for all thromboses (venous and arterial), venous thrombosis (deep and superficial veins), and venous thromboembolism only (deep vein thrombosis with or without pulmonary embolism) for each thrombophilic defect

	Yes	No	Conditional risk ratio (95% CI)	Incidence of thrombosis per 100 patient-years
All thromboses				
No defect	15	312	1 (ref.)	0.15
Antithrombin deficiency	30	55	8.1 (3.4–19.6)	1.0
Protein C deficiency	19	45	7.3 (2.9–18.4)	0.85
Protein S deficiency	15	26	8.5 (3.5–20.8)	1.0
Factor V Leiden	35	165	2.2 (1.1–4.7)	0.29
Venous thrombosis				
No defect	9	312	1 (ref.)	0.09
Antithrombin deficiency	30	55	8.1 (3.4–19.6)	1.0
Protein C deficiency	16	45	7.4 (2.7–20.5)	0.72
Protein S deficiency	12	26	10.4 (3.8–28.7)	0.78
Factor V Leiden	30	165	4.6 (1.5–13.7)	0.25
Venous thromboembolism				
No defect	3	312	1 (ref.)	0.03
Antithrombin deficiency	27	55	42.8 (10.2–180.3)	0.93
Protein C deficiency	14	45	31.3 (7.0–138.8)	0.63
Protein S deficiency	12	26	35.7 (7.9–160.1)	0.78
Factor V Leiden	17	165	10.1 (2.3–43.7)	0.14

Conditional risk ratios are adjusted by sex and family status.

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It is accepted that individuals with thrombophilia and without thrombosis should not be anticoagulated and guidelines for management of individuals without thrombophilia and with a first episode of thrombosis are well established. However, until the immediate past, an answer to the question whether patients with inherited antithrombin, protein C and protein S deficiencies need to be treated after the first episode of thrombosis had been investigated in only a few studies with a limited number of included patients. To determine the high long-term absolute risk of recurrent venous thromboembolism a single-center retrospective study recruited patients from a previously studied large cohort of families with hereditary deficiencies of either protein S, protein C or antithrombin [45]. Ninety-one probands with deficiencies of either protein S (n = 39), protein C (n = 40) or antithrombin (n = 12) were selected because they had already experienced a first episode of venous thromboembolism. Of their 528 relatives that were alive and aged 15 years or older, 468 had qualified for inclusion (224 deficient, 244 nondeficient) and of these 374 had a history of venous thromboembo-

lism (141 deficient, 233 nondeficient) but were not eligible. Annual incidences (95% CI) of recurrent venous thromboembolism were 8.4% (5.8–11.7) for protein S deficiency, 6.0% (3.9–8.7) for protein C deficiency, 10% (6.1–15.4) for antithrombin deficiency and overall 7.7% (6.1–9.5). The authors concluded that patients with hereditary protein S, protein C or antithrombin deficiencies appear to have a high absolute risk of recurrence.

Hypercoagulability

Basal Hemostatic State: Hemostatic Balance

Distribution of blood in a healthy adult male or female is depicted in figure 3 with the major portion of blood residing in the venous system. In blood, the basal hemostatic state is the condition of the balance between clotting, anticlotting and fibrinolysis reactions that maintain blood fluid in arteries and veins. The basal hemostatic state is specific to individual genotypic and phenotypic characteristics that predispose some to an increased ten-

Fig. 3. Distribution of blood at rest.

dency to bleed while putting others at higher risk for arterial or venous thrombosis. Genetic mutations and gene polymorphisms may contribute significantly to changes in the basal state causing a tendency to excess bleeding or hypercoagulability. Thrombophilia may contribute to changes in the basal state that progress to thrombosis [41], thrombosis being the abnormal formation of a fibrin clot in circulating blood.

How do we define hypercoagulability? In a well-balanced hemostatic system, blood levels of coagulation proenzymes regulate checks and balances and are controlled by general catabolic pathways but only to a minor extent by activation events. In circulating blood, activation events, mainly provoked by inflammation, vascular injury, endotoxins, antiphospholipid syndrome, cancer or thrombophilia, release biochemical markers in blood. Elevated levels of biomarkers in plasma indicate hypercoagulability. Hypercoagulability implies, infers, and suggests that one individual has a heightened potential to develop arterial or venous thrombosis when compared to a normal individual of the same age, sex, weight and general health. Hypercoagulability exists in individuals with documented thrombosis who are not anticoagulated (this is the basis for administering anticoagulants); anticoagulation restores the hemostatic balance (anticoagulants do not dissolve clots and by correcting hypercoagulability, they prevent the clot extension and propagation).

Hypercoagulability Is Recognized by Elevated Levels of Biomarkers in Plasma

The United States National Institutes of Health defines a biomarker as a characteristic that is objectively measured and evaluated as an indicator of a normal bio-

logical process. Intravascular clotting is a normal biological process inappropriately triggered in circulating blood mainly by de-encrypted tissue factor, the most potent trigger of coagulation zymogen activations [46]. Since there is minimal activation of clotting mechanisms in the hemostatic state, biochemical markers of activation circulate in blood in restricted amount.

Biochemical Markers of Clotting Reactions, Inflammation and Vascular Damage

Tissue factor, an integral cellular membrane protein, is the receptor and cofactor for factor VII on all somatic cells including endothelial cells. Tissue factor does not circulate in blood under normal circumstances [46–48]. Thrombomodulin, the protein C endothelial cell receptor, is abundant on arterial endothelial cells [49]. Thrombomodulin and soluble tissue factor, byproducts of the proteolytic degradation of their respective receptors, when identified in plasma are biomarkers of vascular injury and inflammation [50, 51]. Fibrinogen is the most abundant coagulation protein [52] (fig. 4) and thrombin proteolysis of fibrinopeptides A and B from fibrinogen produces a soluble fibrin species (fig. 5). Fibrinopeptides A and B, and soluble fibrin (also named thrombin precursor protein), are markers for early thrombin activity [53–55]. Following thrombin release of the transglutaminase activity from factor XIII, D-dimer is a product of plasmin digestion of covalent fibrin [56] and is a marker of both late thrombin activity and plasmin activity [57] (fig. 6, 7). The adhesion molecule P-selectin (CD 62P) resulting from degradation of the P-selectin receptor expressed on activated platelets mediates the rolling of monocytes on activated endothelium and promotes atherosclerotic development, thus soluble P-selectin is a marker of inflammation [58, 59]. A listing of the biomarkers, the time frame of their appearance in relation to thrombin activity, to vascular injury and inflammation as well as their concentration in plasma is given in table 2. Monoclonal antibodies are the tools commonly used to measure levels of biomarkers in blood and plasma. Monoclonal antibodies react with newly uncovered epitopes (neo-antigens) as follows:

(1) Early thrombin activity is indicated by peptide release from precursor coagulation factors: factor Xa releases profragment 1 + 2 from prothrombin; thrombin releases from fibrinogen the fibrinopeptides A and B and the soluble fibrin monomer [51, 53, 54].

(2) Early thrombin activity is indicated by enzyme inhibitor complexes: thrombin-antithrombin complexes appear early in the clotting process [51].

Fig. 4. Native fibrinogen is a symmetric dimeric structure consisting of three pairs of nonidentical polypeptide chains: A α , B β and γ . Factor XIII, α_2 -antiplasmin, plasminogen, and tissue plasminogen activator are attached to fibrinogen. This schematic representation of fibrinogen is adapted from Doolittle et al. [52].

Fig. 5. Fibrinopeptide A (FPA) and fibrinopeptide B (FPB) and soluble fibrin monomer are biomarkers of early thrombin generation. Thrombin cleaves fibrinopeptides A and B from fibrinogen to form des-AA- and des-BB-soluble fibrin monomer. Fibrinopeptides A and B account for 1% of the mass of native fibrinogen. This schematic representation of fibrinogen is adapted from Doolittle et al. [52].

Fig. 6. Soluble fibrin monomers, released from fibrinogen by thrombin removal of fibrinopeptides A and B, noncovalently attach between E- and D-domains. Cross-linking D-domains form insoluble fibrin. D-dimer is a marker of late thrombin activity occurring consequently to factor XIII activation by thrombin [56].

Table 2. Biomarkers of coagulation activation reactions and vascular injury

Biomarker	Baseline plasma concentration	References
Early thrombin activity		
Prothrombin fragment 1 + 2	0.9 ± 0.1 nmol/l	[62–68]
Thrombin-antithrombin complex	2 ± 0.3 μ g/l	[62–68]
Fibrinopeptide A	4.3 ± 0.3 ng/ml	[53, 62–68]
Desarginine fibrinopeptide B	$>1 \pm 0.3$ pmol/ml	[68]
Thrombin precursor protein TpP	2.9 ± 0.7 μ m/ml	[54, 55, 71, 72]
Late thrombin activity		
Cross-linked insoluble fibrin II		
Activated factor XIII		
D-Dimer	5 ± 0.4 μ g/l	[57, 62–68]
Vascular injury and inflammation		
Thrombomodulin	2.90 ± 1.57 ng/ml	[49]
Tissue plasminogen activator antigen	8 ± 1.8 μ g/l	[69]
Tissue factor antigen in venous blood	235 ± 101 pg/ml	[48]
Soluble P-selectin	95 ± 66 ng/ml	[58, 59]

Fig. 7. Cross-linked fibrin undergoes fibrinolysis, the peptides joining D- and E-domains are cleaved, leading to fibrin split products E, D-dimer, D-trimer, D-tetramer, and DY [56].

(3) Late thrombin activity is indicated by plasmin digestion of covalent fibrin with D-dimer release [56, 57].

(4) Components of the vascular endothelium that are not normally present in blood: tissue plasminogen activator antigen, soluble tissue factor and thrombomodulin [48, 49].

(5) Products of platelet activation: splicing of the transmembrane P-selectin platelet receptor releases soluble P-selectin antigen in plasma [58, 59].

Biomarkers in Blood Stasis

Although this has never been confirmed by controlled large population studies, blood stasis is considered the environmental condition most frequently associated with hypercoagulability [60]. Similar to inherited thrombophilia, the hypercoagulability attributed to blood stasis does not necessarily progress to thrombosis. For example, blood stasis is a hallmark of pregnancy, yet half the population of the world becomes pregnant at least once, and only a small fraction is treated for thrombosis. In schistosomiasis, nematodes occlude portal veins causing blood stasis without evidence of thrombosis. It is also not well understood why the hyperviscosity syndromes – polycythemia, myelogenous leukemia, and dehydration – are mostly associated with portal vein thrombosis and retinal vein thrombosis. Atherosclerosis, venous varicosities, incompetent cardiac and venous valves and cardiac fibrillation cause blood stasis but not always thrombosis. Prinzmetal's angina, Raynaud's disease and cryoglobulinemias are associated with vasospasticity but not thrombosis and, paradoxically, the incidence of venous thrombosis is extremely high in complete spinal cord resection consequent to blood stasis [61].

Screening for Hypercoagulability Biomarkers

Indications for hypercoagulability screening based on personal and/or family history of venous thromboembolism would be more cost-effective than universal screening for thrombophilia in all patient groups evaluated; but unlike thrombophilia, the clinical relevance for hyperco-

agulability screening has never been established. Hypercoagulability changes in the hemostatic basal state have been reported in several small studies, but coagulation biomarkers have been mostly studied in the assessment of cardiovascular disease to predict outcomes and to predict atherosclerosis disease progression [62, 63]. Biomarkers of coagulation that define hypercoagulability are elevated after administration of activated factor IX concentrates to arrest bleeding in hemophilia with inhibitor [64] and when elevated in thrombophilia they are an indication of high thrombosis risk [65, 66]. In a selected number of individuals with inherited thrombophilia who have never had a documented thrombosis, thrombosis risk was assessed by molecular coagulation markers [65]. In essential thrombocythemia, polycythemia vera, and in noncardioembolic stroke subtypes moderate levels of late thrombin and fibrinolysis activities and vascular damage, without radiographic evidence for thrombosis, identify a high thrombosis risk [67–69]. Levels of inflammatory and hemostatic biomarkers were measured in the Edinburgh Artery Study, a population cohort study of men and women aged 55–74 years to determine the interplay between inflammatory and hemostatic mechanisms in the development and progression of atherosclerosis [70]. At the start of the study in 1987 and throughout 12 years' follow-up baseline levels of fibrinogen, fibrin D-dimer, von Willebrand factor, tissue plasminogen activator antigen, prothrombin fragment 1 + 2, urinary fibrinopeptide A, C-reactive protein, and interleukin-6 were measured. Peripheral arteriosclerotic progression was assessed by computing ankle brachial index at baseline (1,582 participants) and after 12 years of follow-up (813 participants). Inflammatory markers but not hemostatic factors with the exclusion of fibrinogen and D-dimer were significantly associated with ankle brachial index changes independently of baseline ankle brachial index and other cardiovascular disease risk factors. The data supported the hypothesis that inflammation is more related to atherosclerosis than is hypercoagulation [70]. In a study conducted in Japan, soluble fibrin monomer-fibrinogen complex in plasma derived from patients undergoing surgery and patients with disseminated intravascular coagulation were measured by a monoclonal antibody IF-43 that specifically recognizes thrombin modified fibrinogen (des-AA- and des-AABB-fibrin monomer) [71]. Levels of soluble fibrin monomer were increased at the initial phase of disseminated intravascular coagulation strongly suggesting a role for soluble fibrin monomer as a marker of early thrombin generation [72].

Cases

Case 1: Thrombophilia and Hypercoagulability

A 34-year-old male was the first reported case in 1989 of the second biochemical cause for thrombosis related to the inability of APC to inactivate activated factors V and VIII. APC is the enzyme released from protein C by thrombin/thrombomodulin activation of protein C on endothelial surfaces. There was a family history of thromboses for the mother and sister who both had postpartum thromboses and for the father, who had a thrombosis after gallbladder removal. The patient's history in chronological order is as follows. The first thrombotic episode was at age 23 in 1979, and he was treated with Coumadin, and despite high Coumadin dosages notes in his chart by the hematologist indicated that he was 'immune to it', an assessment made on the basis of minimal prolongation in the prothrombin time (13.1 s, control 11.4 s).

He was prescribed subcutaneous heparin 3 times daily every 8 h but he continued to have ongoing episodic bilateral deep venous thrombosis in his lower extremities and thrombosis in the left forearm. In 1989 he was tested for antithrombin III, fibrinogen and protein C plasma levels at the University of Washington in Seattle, Wash., USA. The plasma levels of the anticoagulants and fibrinogen were found to be normal and he was used as a patient case study to demonstrate that in the year 1989, the cause of thrombosis remained unidentified in 30% of patients with thrombosis. On February 24, 1989 a Hickman catheter was placed in the inferior vena cava through which the patient was administered continuous heparin infusion (1,000 units per hour) via a metafusion system started at the rate of 1 ml/h. On May 4, 1989 the patient was on vacation in Hawaii and while on continuous heparin infusion he developed a severe headache. At the emergency room he was given a sedative and released. He returned a few hours later with generalized seizures and was subsequently diagnosed with sagittal sinus thrombosis and he was flown from Hawaii to the Virginia Mason Clinic in Seattle, Wash. for treatment. On May 14, 1989 he was referred to the author from the Virginia Mason Clinic for diagnosis and management. At the Special Coagulation Laboratory, Michigan State University, results of a diagnostic panel were: fibrinogen measured 2 g/l with no evidence of variant fibrinogen. Antithrombin III heparin cofactor activity was 100% and immunoreactive antithrombin III levels 100%. Factors V and VIII were activated in citrate patient plasma by a silica reagent and phospholipids, while plasma protein C was simultaneously activated by a purified component of *Agkistrodon contortrix contortrix* venom (Protac). Thirty microliters of activated patient plasma was added to 70 µl of factor V genetically deficient plasma and the clotting times of the plasma mixtures were determined in an activated partial thromboplastin assay. An identical experiment was performed with patient plasma added to factor VIII-deficient plasma. The clotting times were 26 and 27.5 s, respectively, indicating that activated factor V and activated factor VIII were not inactivated by APC (pooled normal plasma control prolonged to 68 s in factor V-deficient plasma and to 82 s in factor VIII-deficient plasma). On September 20, 1989 the patient was hospitalized at St. Lawrence Hospital in Lansing Michigan and he was started on a synthetic vitamin K agonist, Miradon (anisindione, indanedione derivative), and heparin was stopped. The vitamin K-dependent factor activities were tested in the patient's plasma 1 week after he had been on full dose Miradon. Results for

factor VII activity, 15%, factor IX, 63%, factor X, 8%, and factor II, 9%, indicated adequate response to the oral anticoagulation. Dilantin and phenobarbital were stopped in early December 1989, and the patient has been on Miradon since September 1989. No further thrombosis has developed and in 1999 genetic testing confirmed a homozygous factor V Leiden mutation.

Case 2: Hypercoagulability without Thrombophilia

A 60-year-old white male was referred for failing 2 lower extremity femoral-femoral bypasses, and an axillofemoral bypass. Of interest in his medical history are two renal transplants, colon cancer treated by chemotherapy and splenectomy and frequent infections by pneumococci and other encapsulated bacteria. He was not a carrier for any thrombophilia mutation or polymorphisms. Results of coagulation studies when the patient was not on Coumadin were: prothrombin time 13.1 s (normal range: 10–12.8 s, control: 11.4 s); activated partial thromboplastin time 30.9 s (normal range: 20–30 s, control: 26.4 s); thrombin clotted fibrinogen 2.3 g/l (normal range: 2–4 g/l); heat-precipitated fibrinogen measured by Lowry's chemical assay 4.5 g/l (normal range: 2–4 g/l). Variant fibrinogen was identified. Mixing studies with an equal volume of pooled normal plasma resulted in prolonged activated partial thromboplastin time corrected to 27.6 s, and a lupus anticoagulant was not identified. All procoagulant factor activities were borderline low: factor II activity 75%, factor V activity 78%, factor VII activity 69%, factor VIII activity 79%, factor IX activity 82%, factor X activity 72%, factor XI activity 86%. All anticoagulant factor activities and antigens were borderline low: antithrombin heparin cofactor activity 68% (normal range: 70–100%), antithrombin immunoreactive protein 70% (normal range: 70–100%), protein C activity (inactivation of factors V and VIII) 73 and 78%, respectively (normal range: 60–100%), protein C immunoreactive levels 78% (normal range: 60–100%), protein S immunoreactive free levels 37%. This profile, compatible with in vivo activation of clotting (low grade disseminated intravascular coagulation), was confirmed by further tests. Plasminogen/plasmin activity by chromogenic substrate assay (250%, normal range: 80–100%) was significantly elevated and clot lysis time (2 min, normal range: 7–12 min) was significantly shortened. Biomarkers of early thrombin and late thrombin activity were measured by monoclonal antibodies raised against prothrombin profragment 1.2, thrombin-antithrombin complex, and fibrinopeptide A. Biomarkers for vascular injury, tissue plasminogen activator (t-PA) and thrombomodulin were measured by monoclonal antibodies and fibrinolysis activity was assessed by monoclonal antibodies specific against D-dimer neoantigen. Results of antibody testing: prothrombin fragment 1.2 was 5.6 nM (baseline 1.1 nM); thrombin-antithrombin complex 6.8 µg/l (baseline 2.0 µg/l) and fibrinopeptide A levels 18.7 pmol/ml (normal range 0.4–4 pmol/ml). There is evidence of hypercoagulability. Biomarkers for vascular injury, t-PA and thrombomodulin were elevated above the normal concentration: t-PA 16 µg/l (normal 8 µg/l) and thrombomodulin 9 µg/l (normal 3.4 µg/l). D-dimer plasma levels were significantly elevated to 5 µg/ml; D-dimer baseline levels were 500 ng/ml. Inherited thrombophilia had been ruled out by genetic testing.

Evidence of early and late thrombin activity (profragment 1.2, thrombin-antithrombin complex and fibrinopeptide B levels were elevated), of vascular damage, t-PA 12 µg/l and thrombomodulin 5 µg/l, and significantly elevated D-dimer (5 µg/ml) lev-

els are indicative of hypercoagulability, a profile compatible with low-grade chronic infection.

Case 3: Thrombophilia without Hypercoagulability

A 79-year-old patient was hospitalized with bilateral chronic deep venous thrombosis of 30 years' duration. A pleasant vivacious, intelligent woman, she was admitted to the hospital at the request of the home care nurse. The nurse visited the patient at home routinely every 3 weeks to check her blood pressure. Elevated blood pressure had been difficult to control for many weeks. Moderate pain was noted on examination of the patient's right calf, which was obviously larger than the left calf. At the hospital, chronic occlusion of venous blood flow (collateral veins) in both calves was detected by Doppler ultrasound. Relevant history: para 2, uneventful pregnancies. No history of thrombosis in any immediate family member. Thirty years before, long after the birth of her last daughter, she occasionally complained of calf pain in one leg, then in the other leg. She suspected she had thrombosis but did not seek treatment. Her undocumented deep venous thrombosis had never progressed to clinical significance. She was not on oral anticoagulant medication at any time during the 30 years that she was mildly symptomatic. She has always been overweight and hypertension is her major concern. She sometimes felt tingling and numbness in one side of her face and tongue but denied a motor deficit. At the hospital, while on loop diuretic and a centrally acting antihypertensive, her systolic pressure was 200 mm Hg and diastolic 160 mm Hg. A tentative diagnosis of thrombophilia was made; she was considered not to have risk of bleeding and, although there had been no report of a new venous occlusion, she was given oral Coumadin 5 mg daily. By genetic testing she was identified as a heterozygous carrier for the factor V Leiden mutation, but as her INR shifted for 2 weeks between 2.3 and 4.5, the risk for bleeding posed by uncontrolled hypertension became too great and Coumadin was stopped. She was tested for hypercoagulability phenotypic changes in plasma 1 week after stopping Coumadin. Prothrombin fragment 1.2 was 1.6 nM (baseline 1.1 nM); thrombin-antithrombin complex 1.8 µg/l (baseline 2.0 µg/l) and fibrinopeptide A levels 0.7 pmol/ml (normal range 0.4–4 pmol/ml). There was no evidence for hypercoagulability and treatment with Coumadin was not resumed.

Conclusion

The intent of this review was to present a chronology of the discovery of acquired and inherited thrombophilia. APS is the first recognition of a thrombophilia tied to venous and arterial thrombosis and recurrent fetal loss. The diagnosis of APS has to include the prolongation of clotting time in phospholipid-dependent assays and the detection of antiphospholipid/β₂-glycoprotein 1 antibodies and the severe type is characterized by systemic generalized thrombotic microangiopathy. The incidence of inherited thrombophilia is 24–37% in individuals with thrombosis compared to a 10% incidence in individuals without thrombophilia and with thrombosis. A large co-

hort family study conducted in Italy supported the evidence first recognized in heterozygous carriers of protein C mutations that individuals with thrombophilia do not necessarily develop thrombosis during their lifetime. Data from another significant study indicates that patients with inherited antithrombin, protein C and protein S deficiencies need to be treated after the first episode of thrombosis. The altered hemostatic state in hypercoagulability is identified by biomarkers of clotting of vascular damage and inflammation. Hypercoagulability is a laboratory phenotype that has been evaluated in numerous

studies and although it is a risk factor for thrombosis, it does not necessarily result in thrombosis. The liberal use of case vignettes emphasized a close relationship as well as the distinction between thrombophilia, hypercoagulability and thrombosis.

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