Hereditary thrombophilia: investigation and management

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ARTICULO ESPECIAL

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I. Differential diagnosis of the patient presenting with thrombosis or a thrombotic diathesis

Inherited (Primary) Hypercoagulable States

Acquired (Secondary) Hypercoagulable States

In association with physiologic or thrombogenic stimuli post-operative state, pregnancy and post-partum, estrogen use, immobilization, trauma, aging

Lupus Anticoagulant or antiphospholipid syndrome

In association with other clinical disorders neoplasia, myeloproliferatigve disorders, PNH, cancer chemotherapy, nephrotic syndrome, heparin-induced thrombocytopenia

Hyperhomocysteinemia

II. Prevalence of defects in patients with venous thrombosis

	%
Activated Protein C Resistance (Factor V-Arg506Gln)	12-40
Hyperhomocysteinemia	10-20
Prothrombin Gene Mutation (G → A transition at position 20210 in the 3'- untranslated region)	96-18
Deficiencies of Antithrombin III, Protein C, Protein S	05-15
Antiphospholipid Antibody Syndrome	10-20
Unknown	15-70

III. Molecular basis of hereditary thrombophilia

	# of Mutations
Activated Protein C Resistance (Factor V Leiden)	1 Dominant
Prothrombin Gene Mutation	1
Antithrombin III Deficiency	> 79
Protein C Deficiency	> 160
Protein S Deficiency	> 69

IV. Physiology of the natural anticoagulant mechanisms

A. ANTITHROMBIN III

1. Primary phyysiologic inhibitor of thrombin; also neutralizes factors IXa, Xa, XIa and XIIa
2. Mechanism of heparin action - accelerates the rate of enzyme (thrombin, factors IXa, Xa, XIa, XIIa) neutralization by antithrombin III
3. In the absence of heparin, antithrombin III (AT III) is a relatively slow inactivator of these activated clotting factors. Anticoagulantly active heparan sulfate species associated with the vascular endothelium are able to accelerate the rate at which antithrombin neutralizes these enzymes.

B. PROTEIN C

1. Vitamin K-dependent protein
2. Converted to a serine protease activated, protein C (APC), by thrombin; rate is accelerated by the endothelial cell receptor protein, thrombomodulin
3. APC is able to specifically inactivate factors Va and VIIIa by limited proteolysis; profibrinolytic effect of APC demonstrable in vitro and in some animal models, but has not yet been established in humans

C. PROTEINS

1. Vitamin K-dependent protein; not a zymogen of a serine protease
2. Exists in plasma in two forms; free (active) and complexed with C4b-binding protein (inactive)
3. Forms a 1:1 complex with APC on platelet membranes and is a cofactor for the expression of APC's anticoagulant activity

V. Assay measurements in heterozygous deficiencies of Antithrombin III and Protein C

Types	Antigen	Functional Activity
I	Low	Low
II	Normal	Low

VI. Assay measurements in Protein S deficiency

	Antigen	Protein S
Total Protein S	Free Protein S	Activity
Low	Low	Low
Normal	Low	Low
Normal	Normal	Low

VII. Clinical features of inherited deficiencies of Antithrombin III, Protein C, and Protein S

Deficiency	Antithombin III	Protein C	Protein S
Prevalence in general population			
Type I	1:5,000	1:200-300	Unknown
Type II	1:200-400		
Symptomatology in homozygotes	Arterial thrombosis in childhood (type II heparin-binding site defects)	Neonatal purpura fulminans in severe cases; DVT/PE starting during adolescence in milder cases	

VIII. Common causes of acquired deficiencies in Antithrombin III, Protein C, Protein S

Antithrombin III	Protein C	Protein S
Neonatal period	Neonatal period	Neonatal period
Pregnancy		Pregnancy
Liver disease	Liver disease	Liver disease
DIC	DIC	DIC
Nephrotic syndrome		
	Chemotherapy (CMF)	
Major surgery states		Inflammatory
Acute Thrombosis		Acute thrombosis
Treatment with:		
Heparin	Warfarin	Warfarin
L-asparaginase	L-asparaginase	L-asparaginase
Estrogens		Estrogens

IX. Warfarin-induced skin necrosis

Clinically: painful, ecchymotic skin lesions in fat containing areas of the body. Occurs during the initiation of oral anticoagulation particularly when large loading doses are used.

Pathologically: skin biopsies show fibrin thrombi within venules with hemorrhagic necrosis.

Incidence: rare occurrence even among protein C deficient patients.

Diagnostically: hereditary protein C deficiency found in 5/16 patients.

X. Hyperhomocysteinemia

A. Atherogeneic and Thrombotic Mechanisms

Direct endothelial cell injury, stimulates smooth muscle cell proliferation

On endothelial cells in vitro, homocysteine
- activates factor V
- inhibits thrombomodulin expression and protein C activation
- impairs generation of nitric oxide and prostacyclin
- suppresses heparan sulfate expression

B. Intracellular metabolism of homocysteine occurs through remethylation to methionine or transulfuration to cysteine. The principal enzymes involved are: (1) methionine synthase, (2) 5,10-methylenetetrahydrofolate reductase MTHFR, (3) betaine-homocysteine methyltransferase, (4) cystathionine-β-synthase.

C. ? A treatable risk factor

Independent risk factor for atherogenesis, MI, stroke, venous thrombosis

Hereditary defects present in many cases

Homozygosity for a thermolabile MTHFR C677T variant (50% active) present in ~ 5% of the general population.

Reduced levels of folate, vitamin B12, or vitamin B6 are associated with hyperhomocysteinemia.

While vitamin supplementation can reduce plasma homocysteine levels, it is unknown whether this reduces the risks of vascular complications.

XI. Resistance to Activated Protein C (APC)

A. Dahlback observed that plasma samples from some patients with thrombotic disease had a poor anticoagulant response to activated protein C (Dahlback B et al, Proc Natl Acad Sci USA 90: 1004-1008, 1993).

B. A defect in factor V involving the mutation of arginine-506 to glutamine-506 (Arg506Gln) is most often the cause of APC resistance. This is the site at which APC cleaves factor Va, and the sequence alteration renders the mutant factor Va molecule relatively resistant to inactivation by APC.

Factor V-Arg506Gln is the cause of APC-resistance in over 80% of Dutch patients (Bertina RM et al., Nature

369: 64-67, 1994). it is only found almost exclusively in Caucasian populations.
C. Combined deficiencies (e.g., Factor V Leiden and antithrombin III, protein C deficiency, protein S deficiency, or the prothrombin G20210A mutation) result in an increased thrombotic risk.

XII. Prothrombin gene mutation (G → A mutation at position 20210 in 3'-unstranslated region)

Population	20210 AG Genotype %
DVT/PE with positive family history	18
Consecutive patients with first DVT	6.2
Controls	2.3

Genotype	Prothrombin Range %	
20210 AG	132	95-178
20210 GG	105	55-156

Mechanism for increased prothrombin level unknown: mutation is near cleavage site to which poly A is added in prothrombin mRNA precursor; ? higher translation efficiency or higher stability of transcribed mRNA.

XIII. Prevalence of the Factor V Leiden (FVL) and Prothrombin G20210A mutations

Population	Factor V Leiden (%)	Prothrombin G20210A (%)
European		
Northern	5-10	1.7
Southern	2-3	3
African, Asian	Extremely rare	

XIV. Sites of thrombosis according to coagulation defect

Abnormality	Arterial	Venous
Factor V Leiden	-	+
Prothrombin G20210A	-	+
Antithrombin III deficiency	-	+
Protein C deficiency	-	+
Protein S deficiency	-	+
Hyperhomocysteinemia	+	+
Lupus Anticoagulant	+	+

XV. Case-control studies of risk factors for venous thrombosis

A. Leiden Thrombophilia Study: A Population Based Patient Case Control Study (Koster T et al. Lancet 1993; Vandenbroucke JP et al. Lancet 1994)
Patients: Inclusion Criteria
345 consecutive outpatients < age 70 referred for anticoagulant treatment to 3 regional thrombosis centers in the Netherlands with a first, objectively confirmed DVT between 1988-1992.
Laboratory evaluation ³ 3 months after discontinuation of oral anticoagulants
Patients: Exclusion Criteria
Patients with known malignancy
Controls: Healthy unrelated control subjects matched for age, sex, absence of a history of venous thrombosis and malignancy
APC resistance found in 64/301 consecutive patients (21%) < age 70 presenting with a first DVT and in 5% of controls.
53 of 64 (84%) of the patients carried Factor V-Arg 506Gln

Age Group (yr)	No (%) with APC resistance
< 25	8 (42)
25-34	11 (21)
35-44	11 (19)
45-54	14 (16)
> 54	20 (24)

Risk and incidence of a first episode of venous thrombosis

	Risk	Incidence/ year (%)
Normal	1	0.008
Hyperhomocysteinemia	2.5 ↑	
Homozygous MTHFR C677T	1	
Prothrombin gene mutation	2.8 ↑	
Oral contraceptives	4x ↑	0.03
Factor V Leiden heterozygotes	7x ↑	0.06
Oral contraceptives + Factor V Leiden	35x ↑	0.3
Factor V Leiden	80x ↑	0.5-1
High Factor VIII: C Level (> 150% of normal)	5x ↑	

B. Ridker et al. (NEJM 1995) Physicians' Health Study
14, 916 healthy men (mean f/u 8.6 years)
Factor V-Arg 506 Gln found in 14 of 121 cases (11.6%) and 6% of controls

Relative risk of first DVT/PE 2.7x ↑
Primary cases 3.5x ↑
Secondary cases (cancer present, post-op) 1.7x ↑
Among men > age 60, Factor V-Arg506Gln found in
8 of 31 (25.8%) with primary DVT/PE
10 of 56 (17.9%) with secondary DVT/PE

XVI. "Counter-intuitive observations" in patients with the Factor V Leiden mutation

A. Absence of interaction with some common risk factors
Physicians' Health Study (Ridker et al. 1995)
Relative risk of first DVT/PE 2.7x ↑
"Idiopathic" 3.5x ↑
Cancer present, post-op 1.7x ↑
B. No increased risk after joint replacement surgery (Ryan et al. 1998)

XVII. Treatment of DVT/PE in patients with a hereditary thrombotic disorder (same as for patients without such a diagnosis)

Heparin
Unfractionated heparin
80 μ/kg bolus followed by 18 μ/kg infusion to maintain APTT in therapeutic range followed by 18 μ/kg infusion to maintain APTT in therapeutic range for at least 5 days of PT in therapeutic range for at least 2 consecutive days
Low molecular weight heparin (adjusted for body weight)
Warfarin
Start on day 1 to achieve an INR of 2.0-3.0, Treat for 6 months

XVIII. Recurrent deep venous thrombosis

Prandoni et al. have provided data that the cumulative incidence of DVT following the cessation of therapy in patients presenting with a 1st episode of symptomatic venous thromboembolism is 24.8% at 5 years and 30.3% at 8 years.

Do Factor V Leiden or Prothrombin G20210A lead to an increased risk of recurrent thrombosis?
Yes

Ridker et al. 1995 U.S. Physicians' Health Study

77 men with a first spontaneous DVT - mean f/u 5.7 years

29% of Factor V Leiden+ patients developed recurrences (7.5%/year) vs 11% of Factor V Leiden- patients (1.8%/ years)

Simioni et al. 1997 Italy

251 Italian patients with a first DVT - mean f/u 3.9 years

40% (13/38) of Factor V Leiden+ and 33% (8/24) of G20210A+ patients developed recurrences vs 14.5% of Factor V Leiden- patients
No

Eichinger et al. 1997 Austria

492 patients with venous thromboembolism - mean f/u 2 years

12.2% of Factor V Leiden+ patients and 8% of G20210A+ patients developed recurrences vs 10.6% in unaffected patients

Lindmarker et al. 1999 Sweden (Durac Trial Study Group)

467 patients with a first DVT or PE - mean f/u 4 years

No increase in risk for either mutation in multivariate analysis

Kearon et al. 1999 Canada

Among 77 placebo patients with a 1st idiopathic DVT and a 29% recurrence risk at 1 year, no increase in recurrence risk for either mutation.

Studies of Factor V Leiden and Prothrombin G20210A Mutation as Risk Factors for Recurrent Venous Thrombosis: Potential Reasons for Differing Results

Data analyzed retrospectively
Differences between studies
No. of patients
Selection criteria - Idiopathic vs secondary
Anticoagulant therapy for 1st event
Duration of followup
Data analysis
Differences between populations
Other unknown genetic risk factors

XIX. Antiphospholipid antibody syndrome

In SLE and other clinical situations (e.g., in association with malignancy, infections, idiopathic), immunoglobulins are present which bind to phospholipids and plasma proteins (b_2-glycoprotein I, prothrombin) in vitro and prolong clotting assays. Prolongation is critically dependent on the maount of phospholipid used in the assay. Usually diagnosed by establishing the presence of a lupus anticoagulant in specialized coagulation assays and/or the presence of elevated titers of cardiolipin antibodies (particularly IgG).

Lupus anticoagulants do not cause bleeding but are associated with an increased risk for venous or arterial thrombosis, recurrent fetal loss, thrombocytopenia, and livedo reticularis.

Increased risk for recurrent DVT in patients with antiphospholipid antibody syndrome warrants consideration of long-term anticoagulation after a 1st thrombotic event.

Schulman et al. 1998 Sweden (Durac Trial Study Group)

412 patients with a 1st or 2nd episode of VTE - mean f/u 4 years

29% recurrence risk and 15% mortality in patients with anticardiolipin antibodies vs 14% and 6%, respectively, in unaffected patients

XX. Arguments against testing for hereditary defects predisposing to thrombosis

No effect on type or duration of initial anticoagulation

No consensus that most hereditary defects confer an increased thrombotic risk

Often erroneous diagnoses are made (especially for antithrombin, protein C, or protein S deficiency)

Absence of proven etiologic association (e.g., arterial thrombosis)

Testing is costly

XXI. Why search for hereditary defects predisposing to thrombosis?

A. Improved understanding of the pathogenesis of thrombosis by patient and physician

B. Identification and counselling of family members at higher risk of thrombosis

C. Availability of specific replacement concentrates (i.e., antithrombin III)

D. Obviate expensive diagnostic tests looking for an occult malignancy

XXII. Proposed screening strategy: characterization of thrombophilic patients

Clinical History	"Weakly"	"Strongly"
Age of onset < 50	-	+
Recurrent thrombosis	-	+
Positive family history	-	+

Deficiencies of antithrombin III, protein C, or protein S are very infrequently identified in "weakly" thrombophilic patients.

XXIII. Screening laboratory evaluation for "stongly thrombophilic" patients

A. Test for resistance to activated protein C
Clotting assay
Genetic test for Factor V-Arg506Gln (Factor V Leiden)

B. Genetic test for prothrombin gene mutation

D. Functional assay of antithrombin III (heparin-cofactor assay)

E. Functional assay of protein C

F. Functional assay of protein S

G. Immunological assays of total and free protein S

H. Clotting assay for lupus anticoagulant/ELISA for antiphosphospholipid antibodies

I. Measurement of fasting total plasma homocysteine (normal range 5-16 μmole/L)

XXIV. Laboratory evaluation of "weakly" thrombophilic patients

A. Test for resistance to activated protein C

B. Measurement of total plasma homocysteine

C. Genetic test for prothrombin gene mutation

D. Clotting assay for lupus anticoagulant/ELISA for antiphosphospholipid antibodies

XXV. Frequency of mutations in thrombophilic patients

Mutation	"Weakly" %	"Strongly" %
Factor V Leiden	19	45
Prothrombin G20210A	6	18
Factor V Leiden + Prothrombin G20210A	1	7

XXVI. Randomized trials of prolonged anticoagulation (INR 2-3) for prevention of recurrent deep venous thrombosis (DVT) or pulmonary embolism (PE)

A. DURAC Study Schulman et al. 1997
Two episodes of idiopathic or secondary DVT/PE
6 month group
- 21% (23/111) recurrence rate at 4 years
- 3% major hemorrhage rate

Indefinite treatment
- 2.6% (3/116) recurrence rate at 4 years
- 9% major hemorrhage rate

B. Kearon et al 1999
First episode of idiopathic DVT/PE treated with 3 months of anticoagulation, then patients randomized to placebo or warfarin for 2 years
Mean followup 10 months
Placebo patients
- 29% (16/77) recurrence rate at 1 year

Warfarin patients
- 1% (1/76) recurrence rate at 1 year
3 major hemorrhages

XXVII. Management of venous thromboembolism

Patient Subgroup	Duration of Therapy
Proximal DVT/PE, no prior thrombotic events	
Transient risk factor	3 months or until risk factor resolved
No triggering risk factor	6 months or long-term
With antiphospholipid antibody syndrome	Long-term
Recurrent DVT/PE	6 months-1 year or long-term

XXVIII. Criteria for longterm oral anticoagulation in patients with venous thrombosis

A. Resolution of triggering risk factor
B. Identification of a prothrombotic state
C. Sites and severity of thrombosis
D. Bleeding risk
E. Resolution of thrombosis
F. Personal factors (occupation, lifestyle)

XXIX. Management of hereditary defects predisposing to thrombosis

Risk Classification	Management
High-Risk	Indefinite anticoagulation
2 or more spontaneous thromboses	
1 spontaneous life-threatening thrombosis	
1 spontaneous thrombosis at unusual sites (mesenteric or cerebral venous)	
1 spontaneous thrombosis in the presence of more than a single genetic defect	
Moderate-Risk	Vigorous prophylaxis during high-risk situations
1 thrombosis with a prothrombotic stimulus asymptomatic	

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