Pathogenesis & Laboratory approach to Thrombophilia

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Abstract. Thrombophilia is a term used for any hypercoagulable state, either inherited or acquired. The former is considered after excluding acquired predisposing causes like trauma, immobility, disseminated intravascular coagulation, pregnancy, and antiphospholipid syndrome etc. It frequently results from interplay of genetic and acquired factors. An individual’s risk for DVT would be determined by the combination of his or her baseline propensity for thrombosis and the magnitude of the acute insult. Inherited hypercoagulable states may be secondary to deficiency of natural clotting inhibitors or elevated procoagulants or increased fibrinolytic factors. Amongst these, activated protein C resistance, is the commonest underlying cause. Testing for thrombophilia is best performed in stages. Highest-yield assays (screening tests) should be performed first and, if positive, should be followed by appropriate confirmatory tests. Cornerstone of initial treatment is heparin, either unfractionated or low molecular weight, followed by oral anticoagulation.

Key words: Thrombophilia, inherited

1. Introduction

Thrombophilia is defined as an increased tendency to thrombosis. It may be acquired or inherited. The latter is considered after excluding acquired predisposing causes like trauma, immobility, DIC, pregnancy and antiphospholipid syndrome etc. Thrombosis may be arterial or venous. Venous thromboembolism (VTE) is the commonest manifestation of a thrombophilic state and approximately 25% of Thrombophilia is detected in over 50% of cases following a first clinical episode of VTE. Inherited hypercoagulable states may be secondary to deficiency of natural clotting inhibitors or elevated procoagulants or increased fibrinolytic factors.

Amongst these, activated protein C (APC) resistance, is the commonest underlying cause and is seen in 20-50% patients with inherited thrombophilia.

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2. Prevalence of thrombophilia

Thrombophilia is lowest in unselected patient population and highest in patients with a personal and/or family history of thrombophilia. Reported prevalence of prothrombin gene mutation and factor V Leiden varies according to geographical distribution, prevalence of both of these mutations is much lower in Asian populations unlike in European populations (2:200-7:100 & 2:100-3:100 respectively) (7). In one cohort family study, the overall incidence of VTE (per 100 patient-years) was found to be 1.07 for antithrombin deficiency, 0.54 for protein C deficiency, 0.50 for protein S deficiency, and 0.30 for activated protein C resistance. Likewise for acquired causes the risk is much higher following hip or knee surgery than during pregnancy, the latter, in turn, poses a much higher risk than prolonged air travel (9).

3. Thrombosis threshold

An individual’s risk for DVT would be determined by the combination of his or her baseline propensity for thrombosis and the magnitude of the acute insult. In the face of increased baseline hypercoagulability (e.g., factor V Leiden) even a relatively weak insult (e.g., blood stasis during a flight) can be sufficient to precipitate DVT (10). Likewise, in an individual...
with a relatively low level of baseline genetic hypercoagulability (e.g., a single mutation that is associated with a low risk of thrombosis) a relatively strong thrombogenic event (e.g., pregnancy) would be required to provoke an episode of VTE. Thus, the precipitating event in such individuals is often clinically overt. In most cases, such thrombophilic individuals never suffer VTE throughout their lifetimes, and when they do have an episode, it is unlikely to recur. In contrast, an individual with a high level of baseline genetic hypercoagulability (with multiple thrombophilic mutations or polymorphism) is at such high risk that relatively minor acquired triggers can initiate a thrombotic episode. These triggers are therefore subclinical, giving the appearance that the patient has “idiopathic”, “spontaneous”, or “unprovoked” VTE. Furthermore, VTE in these high-risk individuals is more likely to recur (9).

4. Aetiology of thrombophilia

Hypercoagulable state or thrombophilia arises when there is an abnormality in blood coagulability which is the result of interplay of acquired and inherited factors or several inherited factors.

Table 1. Causes of Hypercoagulable state/Thrombophilia

<table>
<thead>
<tr>
<th>Primary hypercoagulable states (thrombophilias)</th>
<th>Acquired hypercoagulable state</th>
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</thead>
<tbody>
<tr>
<td>1. Decreased antithrombotic proteins</td>
<td>1. Vascular disorders</td>
</tr>
<tr>
<td>Antithrombin deficiency</td>
<td>Atherosclerosis</td>
</tr>
<tr>
<td>Protein C deficiency</td>
<td>Diabetes</td>
</tr>
<tr>
<td>Protein S deficiency</td>
<td>Prothrombin gene mutation G20210A</td>
</tr>
<tr>
<td>2. Increased prothrombotic proteins</td>
<td>2. Abnormal rheology</td>
</tr>
<tr>
<td>Factor V Leiden (activated protein C resistance)</td>
<td>Stasis (immobilization, surgery, congestive heart failure)</td>
</tr>
<tr>
<td>Prothrombin gene mutation G20210A</td>
<td>Hyperviscosity (polycythemia vera, Waldenstrom macroglobulinemia, acute leukemia, sickle cell disease)</td>
</tr>
<tr>
<td>Increased levels of factors VII, XI, IX, VIII, von Willebrand factor</td>
<td>3. Other disorders associated with hypercoagulability</td>
</tr>
<tr>
<td>Cancer (Trousseau syndrome)</td>
<td>Cancer chemotherapy agents, thalidomide</td>
</tr>
<tr>
<td>Oral contraceptive, estrogen therapy, selective estrogen receptor modulators</td>
<td>Pregnancy</td>
</tr>
<tr>
<td>Infusion of prothrombin complex concentrates</td>
<td>Thrombotic thrombocytopenic purpura</td>
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<tr>
<td>Nephrotic syndrome</td>
<td>Disseminated intravascular coagulation</td>
</tr>
<tr>
<td>Myeloproliferative disorders</td>
<td>Antiphospholipid antibody syndrome</td>
</tr>
<tr>
<td>Paroxysmal nocturnal hemoglobinuria</td>
<td>Heparin-induced thrombocytopenia/thrombosis</td>
</tr>
</tbody>
</table>

Approach to thrombophilia

Clinical presentation and diagnosis

- significant percentage of women associated with thrombosis related to pregnancy or oral contraceptive use, have an inherited disorder
- inherited thrombotic disorders are usually associated with venous thromboembolism

History and physical examination

Complete history taking is an essential part of evaluation of patient with thrombosis and should include:

- age of onset
- details about the circumstances proximate to the thrombotic event like surgery, trauma, pregnancy, immobility, estrogen therapy etc.
- women should be asked about any bad obstetric history, hormone replacement therapy and use of oral contraceptive use
- details of any prior thrombotic event.
Table 2. The important pointers for inherited thrombophilia are

<table>
<thead>
<tr>
<th>Most common</th>
<th>Less common</th>
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<tbody>
<tr>
<td>Thrombosis at a younger age</td>
<td>Recurrent pregnancy loss</td>
</tr>
<tr>
<td>Recurrent thrombosis</td>
<td>Pre-eclampsia – HELLP</td>
</tr>
<tr>
<td>Family history of thrombosis</td>
<td>Vitamin K antagonist-induced skin necrosis</td>
</tr>
<tr>
<td>Thrombosis in an unusual site</td>
<td>Neonatal purpura fulminans</td>
</tr>
</tbody>
</table>

- detail family history as history of thrombosis in first degree relative strongly suggest a hereditary defect.
- presence of constitutional symptoms, as thrombosis may be the first manifestation of a malignancy
- presence of an underlying disease like cancer, collagen vascular disease, myeloproliferative disease, atherosclerosis, nephrotic syndrome etc
- intake of drugs like hydralazine or procainamide
- History of recurrent thrombosis in spite of oral anticoagulation which suggests presence of occult neoplasm or recurrent cancer.

Physical examination should be directed to the
- Vascular system
- Extremities
- Chest
- Heart
- Abdominal organs

Laboratory approach to thrombophilia

Whom do we investigate for inherited thrombophilia?
- Young patients with age <45 years with recurrent thrombosis
- Young patients with a single thrombotic event but has a positive family history
- Thrombosis in unusual sites
- Heparin resistance
- Warfarin-induced skin necrosis
- Thrombosis occurring with estrogen therapy or pregnancy

When to investigate for inherited thrombophilia?

- Heparin therapy may be associated with up to 30% reduction in plasma level of ATIII levels over several days; warfarin produces marked reduction in functional protein C and protein S and rarely elevates the level of ATIII. Therefore testing is done when the patient has fully recovered from the acute event and is off anticoagulant therapy. Ideally these tests are carried out 2 weeks after completing initial 3-6 months of anticoagulant therapy. Normal levels during the acute phase effectively exclude deficiency of these proteins.

Laboratory tests

- Complete blood count for evidence of polycythemia vera, essential thrombocytopenia, PNH and heparin-induced thrombocytopenia
- Examination of the peripheral smear for evidence of schistocytes (to rule out disseminated intravascular coagulation, thrombotic thrombocytopenic purpura / hemolytic uremic syndrome). Leucoerythroblastic picture suggests involvement of bone marrow by tumour.
- Liver and renal function tests and urinalysis: Patients with Budd-Chiari Syndrome & Nephrotic syndrome respectively.
- Baseline coagulation tests including PT, APTT for evidence of lupus anticoagulants.
- Tests for thrombophilia

Testing for thrombophilia is best performed in stages. Highest-yield assays (screening tests) should be performed first and, if positive, should be followed by appropriate confirmatory tests.
Table 3. Recommended laboratory evaluation for patients suspected of having an underlying hypercoagulable state

<table>
<thead>
<tr>
<th>Activated protein C resistance</th>
<th>Factor V Leiden PCR</th>
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<tr>
<td>Activated protein C resistance</td>
<td>Factor V Leiden PCR</td>
</tr>
<tr>
<td>Antithrombin, protein C, and protein S activity (functional) levels</td>
<td>Factor V Leiden PCR</td>
</tr>
<tr>
<td>Antigenic assays for antithrombin, protein C, and protein S</td>
<td>Factor V Leiden PCR</td>
</tr>
<tr>
<td>Screening tests for lupus anticoagulant (sensitive aPTT, aPTT mixing studies, dilute Russell viper venom time)</td>
<td>Factor V Leiden PCR</td>
</tr>
<tr>
<td>Fasting total plasma homocysteine level</td>
<td>Factor V Leiden PCR</td>
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<tr>
<td>Prothrombin G20210A mutation testing by PCR</td>
<td>Factor V Leiden PCR</td>
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</tbody>
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**Protein C deficiency**

- Inherited deficiency of protein C a vitamin K dependent anti coagulant protein, which inactivates activated FV and FVIII. Homozygous deficiency presents as life threatening neonatal thrombosis or purpura fulminans. Heterozygous deficiency is more common and is seen in 1 to 5% of general population.

Normal plasma concentration is 4 μg / ml. The logarithm of the values for protein C antigen determined in the normal adult population is usually in the range of 70-140%. Levels <55% are likely to be associated with genetic defect. Protein C levels in newborns are 20-40% of adult levels. The estimated increased lifetime relative risk of venous thrombosis has been reported to be 31-fold. (16) Protein C activity less than 0.68 U/ml, measured at the time of thrombosis in individuals without a known hypercoagulable state, has been associated with increased rates of recurrent venous thrombosis.

**Protein S deficiency**

- Protein S exists in the plasma in two forms: bound to C4b-binding protein (60% of total protein S) and free (40% of total). The total protein S antigen in normal adults is 23μg/ml. Levels increase with increasing age.

Up to 25% of patients with protein S deficiency may experience arterial thrombosis including stroke (17). The estimated lifetime increased relative risk of thrombosis has been reported to be as high as 36-fold for protein S deficiency.

**Type I protein S deficiency**

- Inherited deficiency of protein C a vitamin K dependent anti coagulant protein, which inactivates activated FV and FVIII. Homozygous deficiency presents as life threatening neonatal thrombosis or purpura fulminans. Heterozygous deficiency is more common and is seen in 1 to 5% of general population.

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**Diagnosis of protein S and C deficiency**

- Patients should not be diagnosed as protein S deficient until APC-R is excluded.

- Patients with heterozygous protein C & S deficiency have normal PT and aPTT values whereas those of homozygous protein C deficiency have abnormal coagulation tests consistent with DIC.

Pro C® Global test (Dade Behring, Maburg, Germany) is an effective screening test for protein C and protein S deficiency. It is a coagulometric test based on activation of protein C of test sample by Protein C activating protease from venom *Agkistrodon contortrix*. The resulting prolongation of the clotting time then reflects the functioning of the Protein C anticoagulant pathway [13]. The time taken for clot formation is determined as PCAT (Protein C Activity dependent Clotting Time). This assay is recommended as screening for F V Leiden related APCR, Protein C, Protein S & AT III deficiencies.

**Protein C assay** Functional (chromogenic) & antigenic(ELISA) assays are available.

The **Protein S assay** both functional and immunologic assays are available. Functional assay may be PT-or aPTT-based, measuring inhibition of factor Va by APC this measures free
protein S activity. ELISA measures total protein S levels.

**AT III deficiency**

- **Deficiency of AT III,** which inactivates thrombin, is found in 0.2% general population. It has an autosomal dominant inheritance and constitutes 5-10% of DVT patients.
- **Type I** of inherited ATIII deficiency is a result of reduced synthesis of biologically normal protease inhibitor molecule and type II results from molecular defect within the protease inhibitor. Diagnosis is made by immunologic(ELISA) and functional assays.

  Functional assays assess quantitative as well as qualitative abnormality of ATIII and can be done either by coagulation or by chromogenic methods.

<table>
<thead>
<tr>
<th>Antithrombin III</th>
<th>Protein C</th>
<th>Protein S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonatal period</td>
<td>Neonatal period</td>
<td></td>
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<tr>
<td>Pregnancy</td>
<td></td>
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<tr>
<td>Liver disease</td>
<td>Liver disease</td>
<td>Liver disease</td>
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<tr>
<td>DIC</td>
<td>DIC</td>
<td>DIC</td>
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<tr>
<td>Nephrotic syndrome</td>
<td>Chemotherapy (CMF)</td>
<td></td>
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<tr>
<td>Major surgery</td>
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<td></td>
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<tr>
<td>Acute thrombosis</td>
<td>Acute thrombosis</td>
<td></td>
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<tr>
<td>Treatment with heparin, L- asparginase</td>
<td>Treatment with warfarin, L- asparginase</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Causes of acquired deficiencies in antithrombin III, protein C, and protein S

The normal range of ATIII is between 0.75 and 1.25 IU/ml in adults; in ATIII deficiency, it is less than 0.7 IU/ml. Healthy newborns have concentrations lower than the in adults (0.6 to 0.8 IU/ml) and reach adult level by age 6 months. In ATIII deficient neonates, the level has been found to be less than 0.3 IU/ml.

A variety of pathophysiologic conditions can reduce the concentration of ATIII, Protein C and Protein S in the blood, which one should keep in mind while evaluating their deficiencies. (TABLE 4)

**Activated protein C resistance (APC-R)**

Activated Protein C Resistance (APC-R) is the most common laboratory abnormality in patients with history of thromboembolism. Factor V Leiden accounts for 92% of cases of APC-R; rest 8% is due to pregnancy, OCP use, antiphospholipid antibody syndrome, plasma glucosylceramide deficiency and other factor V mutations. Heterozygous carriers of FVL have a 2- to 10-fold increased lifetime relative risk of developing VTE. This is further increased by pregnancy (9-fold), OCP (36-fold) and HRT (13-16-fold) (8). Homozygous carriers are estimated to have an 80-fold increased lifetime relative risk of VTE. (19).

Specific clinical associations with APC-R:

- Recurrent miscarriages: 20% of second trimester pregnancy loss is associated with APC-R
- Children with thrombosis
- Cerebral venous thrombosis
- Myocardial infarction in young women; risk increases to 30-fold if associated with smoking

Laboratory evaluation of APC-R is done by clotting assays based on inhibition of factor Va by APC and prolongation of clotting time. In individuals with APC resistance, there is very little prolongation of APTT since FVa is not inactivated due to mutation in FV cleavage site leading to failure of APC to recognize it. APC is added to patient plasma, and a clotting assay is performed (PTT), with results expressed as a ratio of patient and normal:

$$\frac{\text{Patient aPTT} + \text{APC}}{\text{Patient aPTT} - \text{APC}}$$

APC-R can also be done by ELISA.

APCR may vary due to variation in reagents/laboratories it is therefore advocated that it be expressed as normalized APC ratio (nAPCR), wherein APC ratio of normal is performed in pooled plasma from at least 20 normals. This improves its accuracy and precision. nAPCR= APC ratio of patient/APC ratio of normal.
APC resistance is said to be present when nAPCR < 0.76; if found positive, molecular studies may be done for FV Leiden mutation. APCR may be due to increased FVIII/V levels, inhibitors to APC or mutation at any of the 3 cleavage sites of FVa. In order to ensure specificity of APCR for FV mutation defects, it is advisable to use FV deficient plasma in a ‘Modified Dahlback’s test’ wherein FV deficient plasma increase the specificity of the test for FV mutations. In cases with APCR, diagnosed by modified Dahlback’s test, Polymerase chain reaction for FV leiden may be performed.

Prothrombin gene mutation

- Recently, a genetic defect in prothrombin gene leading to prothrombin 20210 G → A mutation has been found in 1-2% of normals. As a result of this mutation, these patients have FII levels > 115IU/dl which increases the relative risk of VTE 2-5 times, further increases with pregnancy (15fold), OCP use (16fold)
- PG20210 A can be detected by molecular PCR technique, which can be performed despite concomitant anticoagulant therapy.

Hyperhomocysteinemia

- Hyperhomocysteinemia This may be due to mutations in MTHFR gene or due to deficiency of vitamin B12, B6, or folic acid.

  Inherited severe hyperhomocysteinemia result from homozygous MTHFR and CBS deficiency or inherited errors of cobalamin deficiency. Inherited mild to moderate hyperhomocysteinemia results from the thermolabile variant of MTHFR (tMTHFR) that is encoded by the C677T gene polymorphism.

  Acquired hyperhomocysteinemia result from folic acid deficiency, vitamin B6 and vitamin B12 deficiency, renal insufficiency, hypothyroidism, inflammatory bowel disease, advanced age, carcinoma of the breast, ovaries, pancreas, ALL, drugs like methotrexate, theophylline, phenytoin.

Hypercysteinemia (plasma level > 18.5 micromol/L) has been associated with 2-4-fold increased VTE risk. For evaluation fasting total plasma homocysteine is measured first (normal level 5-15 μmol/L) followed by testing the same 2-8 hours after an oral methionine load (100mg/kg); the later increases the sensitivity of occult B6 deficiency and obligate heterozygotes for CBS deficiency. Vitamin B12 and folic acid do not affect post-methionine homocysteine levels. With raised homocysteine level vitamin B12 level should be measured before starting treatment.

Elevated F VIII levels (>150 IU/dl) confer a 6-fold increased risk of DVT. Increased thrombosis in patients with elevated FVIII levels is often seen in patients with O Blood group and vWF levels > 150 IU/dl.

Conclusion

Investigation for idiopathic thrombophilia is essential for appropriate prophylactic and therapeutic anticoagulation, though only 30-40% have an underlying inherited cause 60% are idiopathic.

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