Hypercoagulability/Thrombophilia Testing

Background Information

Venous thromboembolism (VTE) is a major health issue, with more than 300,000 first-lifetime cases per year and around 1 million deaths annually in the United States alone. Thrombophilia (or hypercoagulability), although not a disease itself, is a major contributing factor in the development of VTE. Thrombophilia is the propensity to develop thromboses due to an acquired or inherited defect in the coagulation system. The predominant clinical manifestation of thrombophilia is venous thromboembolism.

Anti-phospholipid antibody syndrome (APS) is the most common cause of acquired thrombophilia. Additional causes include acquired or inherited deficiency of anticoagulant or procoagulant factors (e.g., protein C, protein S, antithrombin or fibrinogen), acquired or inherited elevation in procoagulant factors such as factor VIII or homocysteine (>95th percentile). Inherited genetic mutations including Factor V Leiden [FVL] and prothrombin gene also predispose to thrombosis.

Not all abnormalities are associated with thrombophilia. For example, thrombophilic risk factors include advancing age (>50), major surgery, trauma, immobilization, malignancy, pregnancy, prior to oral contraceptive or hormonal replacement therapy, and chemotherapy. As with many disease-modifying risk factors, thrombophilic risk factors are synergistic — a combination magnifies the risk for thrombosis.

Acquired fibrinogen deficiency can occur in liver disease, disseminated intravascular coagulation (DIC) or hyperfibrinolysis. Acquired protein C or protein S deficiency can be associated with liver disease, anticoagulant therapy (warfarin), acute thrombosis, infections, DIC, postoperatively, uremia or chemotherapy. Acquired antithrombin deficiency can be associated with DIC, liver disease, heparin therapy, acute thrombosis, nephrotic syndrome, or L-asparaginase therapy.

Currently, there is no single laboratory test that can identify all hypercoagulable defects. Therefore, a combination of laboratory analyses is needed to accurately identify thrombophilic patients. Many of these tests are affected by other — often concurrent — clinical conditions so that the correct interpretation of these specialized laboratory test results can be complicated and always require clinical correlation.

Clinical Indications

- Patients with a personal or family history of unexplained or recurrent thrombosis and/or pregnancy complications.
- Potential benefit for screening patients who will be placed at increased risk of thrombosis.

Interpretation

- This panel of tests is not simply reported as positive or negative. A narrative interpretation is issued for each patient panel.
- Each test is reported separately, taking into account the patient's clinical context.
- Each positive test result increases the relative risk of thrombophilia independently of the other test results.

Limitations of the Assays

Results from a hypercoagulability work-up are difficult to interpret in the setting of acute thrombosis or anticoagulant medication therapy; thus, testing should be performed approximately 30 days after VTE or discontinuation of medication including warfarin, heparin, direct thrombin inhibitors (DTIs) and fibrinolytic agents.

Certain other clinical conditions (e.g. pregnancy, inflammatory states, liver disease, etc.) may affect certain assay results as well. The test requestor should provide appropriate clinical information in regards to these conditions to assist the laboratory in making the best possible interpretation of results. Alternatively, thrombophilic testing may be delayed until these clinical conditions have subsided.

Certain more rare thrombophilic mutations do exist for which testing is not currently performed. In this case, a patient may have an apparently negative thrombophilic work-up while still exhibiting a thrombotic phenotype. Clinical judgment is necessary to guide the therapy of these patients.

Methodology

Laboratory testing for thrombophilia consists of a panel of assays specifically performed together to maximize diagnostic potential (see figure).

Functional testing:

- *Anti-phospholipid antibody (APA, lupus anticoagulant):* Automated and manual aPTT, and the hexagonal phase phospholipid dependence assay.
- *Protein S:* A turbidometric clot-based assay. If a deficiency is suggested, an antigen level can be measured for confirmation.
- *Protein C and antithrombin:* Chromogenic substrate assays in which the normal ability to cleave substrate molecules causes a color change. If a deficiency is suggested, an antigen level can be measured for confirmation.
- *Activated protein C resistance (APC; a surrogate for the FVL mutation):* An aPTT-based assay using the ratio of APTTs with and without additional APC. If the ratio is decreased (<2), molecular testing is used as confirmation of FVL mutation.
- *Homocysteine levels:* Chemiluminescence immunoassay. While the methylenetetrahydrofolate reductase (MTHFR) gene mutation may be confirmed by molecular methods, this usually is considered unnecessary.
- *Fibrinogen:* Clauss variation of the thrombin time assay (clot-based).
- *Factor VIII:* Clot-based assay.
- *C-reactive protein:* Levels assist in determining whether Factor VIII and fibrinogen are elevated as part of an acute phase response.

Antigenic testing:

- Specific antibodies against cardiolipin by ELISA assay. If positive, antibodies against ß2 glycoprotein 1 are measured.
- Protein S (free and total) antigenic testing may be performed to confirm and/or subtype a deficiency detected by a decrease in protein S functional activity.
- Protein C antigen level may be measured to confirm and/or subtype a deficiency detected by a decrease in protein C functional activity.
- Antithrombin antigen level may be measured to confirm and/or subtype a deficiency detected by a decrease in antithrombin functional activity.

Genetic/Molecular testing:

- A single nucleotide polymorphism (SNP) in a regulatory region of the prothrombin gene (G20210A) accounts for most cases of elevated prothrombin. This SNP is assayed by fluorescence melt-curve analysis.
- FVL may be confirmed (after a decreased APC-R result) by fluorescence melt-curve analysis.

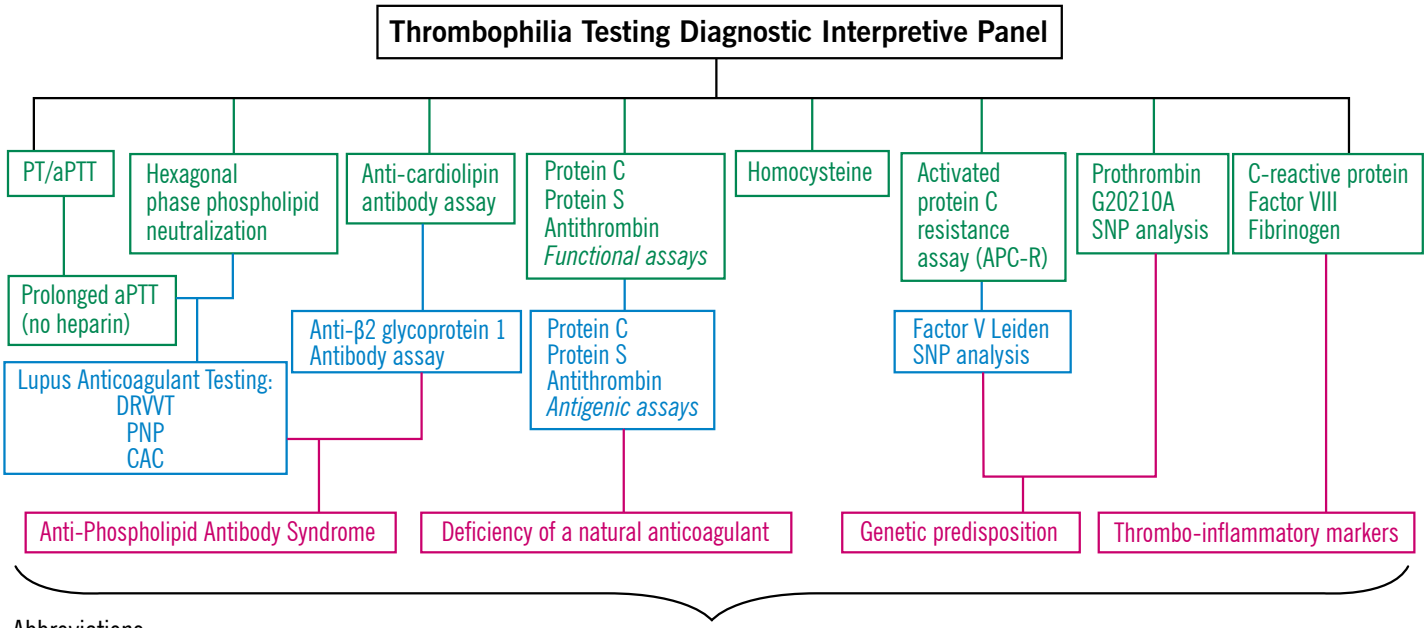
References

1. Colman RW *et al. Hemostasis and Thrombosis: Basic Principles and Clinical Practice*, 5th Ed. Lippincott Williams and Wilkins (2006).
2. Heit J. Thrombophilia: Common Questions on Laboratory Assessment and Management. *Hematology.* 2007;127-35.
3. Kottke-Marchant K. *An Algorithmic Approach to Hemostasis Testing*. CAP Press (2008).

Initial Core Panel Laboratory Testing

Reflex testing, depending on Core Panel results

Thrombophilia risk factors



Patient-Specific Narrative Interpretation

based on composite test results

Abbreviations:

PT - prothrombin time

aPTT - activated partial thromboplastin time

DRVVT - dilute Russell's viper venom test

PNP - platelet neutralization procedure

CAC - circulating anticoagulant assay (mixing study)

SNP - single nucleotide polymorphism

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Test Overview

Test Name	Hypercoaguability testing
Reference Range	See individual test
Specimen Requirements	1. Serum (1 mL) in SST (Gold) 2. Plasma (2 mL) in EDTA tube (Lavender) 3. Whole blood (4 mL) in EDTA tube (Lavender) 4. Plasma (6 mL) in Sodium citrate tube (Lt. Blue)
Special Information	• Patient Preparation: Discontinue warfarin therapy for 7 days, heparin therapy for 2 days and thrombolytic therapy for 7 days prior to test, if possible. • Submit a Coagulation Consultation Patient History Sheet. • 3.2% sodium citrate is the preferred anticoagulant recommended by CLSI. • If tests are abnormal in the panel, the following tests may be ordered and billed: Circulating Anticoagulant (85732); Dilute Russell Viper Venom (85613); Platelet Neutralization (85597); Factor V Leiden (83891, 83898, 83896 x 2, 83903, 83890); MTHFR by PCR (83890, 83892, 83898, 83894, 83912); Thrombin Time (85670); Reptilase (85635); Fibrinogen Antigen (85385); Prot C Immunologic (85302); Prot S Immunologic (85306); Heparin fXa Inhibition (85520)
Billing Code	173
CPT Codes	83090; 83891; 83896 (x2); 83898; 83903; 83912; 85240; 85300; 85303; 85306; 85307; 85384; 85390; 85610; 85730 (x2); 85732; 86140; 86147 (x3)

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