



## 49. Thrombophilia laboratory testing

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**Q1: "I am hetero FVLeiden (female, 50 years old) and had a PE one year ago. I have not been tested for any other clotting problems other than homocysteine (which was normal). Should there be any other tests?"**

A1: If this patient had a spontaneous PE without temporary risk factors (surgery, trauma, or immobilization), I would do additional thrombophilia work-up. Further testing would help me determine whether the patient has a high risk for recurrence of DVT or PE and whether I might recommend long-term full-dose (INR 2.0-3.0) or low-dose (INR 1.5-2.0) coumadin® therapy.

**Q2: "My sisters have both been diagnosed with factor V Leiden hetero. One sister had one miscarriage and two stillbirths (one at 7 mo. and the other at 6 mo.). The other sister had one miscarriage and three normal pregnancies. What could be the difference between my two sisters to have such different outcomes in their pregnancies?"**

A2: Pregnancy loss (as well as thrombosis) is a multi-factorial problem: several risk factors come together to cause the pregnancy loss (or the thrombosis). Factor V Leiden is only one of the risk factors. The one sister probably has more risk factors for pregnancy loss than the other does.

**Q3: "I have factor V Leiden and am positive for deficits in proteins C and S."**

A3: To have protein C and S deficiency at the same time is extremely rare. Whenever I hear that a patient has been found to have such a combined "deficiency" I first suspect that (a) the patient was tested when he/she was on warfarin (warfarin makes protein C and S levels go down), (b) the test is not valid because the blood draw was bad, the sample was not handled appropriately, or an inferior quality lab performed the test.

**Q4: "Hang on to your chairs folks---my daughter was recently tested for clotting disorders...the bill came....FOR \$1200.00!!!!!!!!!!!!Aaaaaaggghhhhhhhhh!!!!!!!!!!!!!! Can you imagine? She has no insurance...."**

A4: Genetic testing and coagulation testing are, indeed, quite expensive. This should be taken into consideration and discussed by physician and patient, when considering extensive testing to look for a clotting abnormality.

**Q5: "My antithrombin activity was high. What could that mean? My hematologist never mentioned it to me."**

A5: Elevated levels of antithrombin III, protein C, and protein S have no known clinical significance. Most, if not all, hematologists therefore do not pay any attention to them. Theoretically, high levels could cause a bleeding tendency, but such an association has, to my knowledge, not been looked for.

**Q6: "Is it possible for the protein S deficiency to skip a generation? I have heterozygous FVL but no protein S deficiency. My son has both. Is it possible that the lab that did my blood test made a mistake?"**

A6: Neither protein S deficiency, nor factor V Leiden "skip a generation". There are several possible explanations for the above family:

- the son does not have protein S deficiency but the low level is a lab error (see discussion below),
- the son was taking coumadin at the time of testing,
- the son inherited protein S deficiency from his father (and the factor V Leiden from his mother),
- the son does not have inherited, but acquired protein S deficiency (such as can be seen in liver disease, nephrotic syndrome, infections),
- a lab error occurred.

Many different opinions exist as to what constitutes an appropriate laboratory work-up in a patient who has had a blood clot. Some physicians test for everything imaginable, others for nothing. More extensive testing is not necessarily better than less extensive testing, because

- because positive tests may create uncertainties (in patients, families and physicians) about the implications of the findings;

- because of the possibility of future health- or life-insurance discrimination, if a patient is found to have a thrombophilic abnormality;
- because of the cost of testing to the individual and to the health care system.

My decision to perform certain thrombophilia tests is determined by one or several of the following 4 factors, listed in descending order of importance:

- Does the test result influence the patient's management? i.e.:
  - the length of coumadin therapy if the patient has had a thrombosis,
  - the decision to advise against contraceptives or hormone replacement therapy,
  - the decision to advise giving heparin prophylaxis during pregnancy or postpartum.
- Does a positive test result have consequences for other family members, i.e.
  - should other family members be tested if the patient has an abnormal test,
  - should the other family member be managed differently, if he/she were found to have a thrombophilic abnormality?
- The patient wants to know why he/she developed a clot (even though the test result will not have any bearing on the patient's treatment);
- I want to understand why the patient developed a clot.

If the patient and I decide on thrombophilia testing, then I often obtain the tests listed below. However, individual decisions need to be made, depending on the patient's unique circumstances under which he/she developed thrombosis.

- A. In deep vein thrombosis (DVT) or pulmonary embolism (PE), triggered by surgery, trauma, or immobilization I often test:
  - nothing
- B. In spontaneous DVT or PE, not triggered by surgery, trauma, or immobilization:
  - a. "Standard tests":
    - factor V Leiden genetic test
    - prothrombin 20210 mutation genetic test
    - protein C activity
    - protein S activity and total and free protein S antigen
    - antithrombin III activity
    - antiphospholipid antibodies, consisting of:
      - lupus anticoagulant
      - anticardiolipin IgG and IgM antibodies
    - homocysteine level
    - full blood count (with hemoglobin and platelet count)
  - b. If there is a strong family history of DVT or PE, I may also obtain:
    - thrombin clot time
    - fibrinogen activity
    - fibrinogen immunoelectrophoresis
    - plasminogen activity
  - c. If there is an unusual venous clot, such as Budd-Chiari-syndrome, or mesenteric or portal vein thrombosis, I may also obtain
    - CD55/59 flow cytometry for PNH (= paroxysmal nocturnal hemoglobinuria)
    - Bone marrow biopsy and cytogenetics to look for myeloproliferative disorder)
  - d. Not infrequently I also obtain the following tests, to more completely understand why a patient clotted:
    - fibrinogen activity
    - factor VIII activity level
    - factor IX activity
    - factor XI activity

Elevated levels of these 4 clotting factors have been shown to be associated with an increased risk of venous blood clots. Since management of the patient usually does not differ, even if one finds an elevated level, I do not consider these tests "standard".
  - e. I do not obtain the thermolabile MTHFR (= methylene-tetrahydrofolate-reductase) genetic test, since the data from studies have, in my assessment, not shown that MTHFR is a risk factor for DVT or PE.
  - f. Sometimes I obtain anti-beta-2-glycoprotein-I antibodies, if I am suspecting antiphospholipid antibody syndrome. However, this test is, at present, poorly standardized, and one has to interpret results with

caution.

- C. In unexplained arterial blood clots in young patients (stroke, heart attack, occlusion of an artery in leg or arm), I often obtain the following thrombophilia tests:
- lipid profile (i.e. LDL, HDL, triglycerides)
  - lipoprotein(a)
  - protein C activity
  - protein S activity and total and free protein S antigen
  - antithrombin III activity
  - antiphospholipid antibodies, consisting of:
    - lupus anticoagulant
    - anticardiolipin IgG and IgM antibodies
  - homocysteine level
  - factor V Leiden only in young women who smoke
  - I do not obtain the thermolabile MTHFR (= methylene-tetrahydrofolate-reductase) genetic test, since MTHFR is not a risk factor for arterial thrombosis.
  - I typically do not obtain the prothrombin 20210 mutation since it is not associated with arterial thromboembolism.
- D. In pregnancy loss before week 12 (1 or 2 miscarriages), I usually do not obtain a thrombophilia work-up.
- E. In recurrent pregnancy loss before week 12 (3 or more miscarriages), I usually obtain the following tests:
- antiphospholipid antibodies, consisting of:
    - lupus anticoagulant
    - anticardiolipin IgG and IgM antibodies
  - homocysteine level
- Studies about the role of other thrombophilic abnormalities in causing early pregnancy loss have yielded conflicting results, so that the role of these abnormalities is not clear. I make individual decisions on whether to test or not.
- F. In loss of pregnancy between week 12 and 20 (one loss or more) I do not have a consistent approach, but make individual decisions after discussion with the patient, since the role of thrombophilia is not clear yet.
- G. In loss of pregnancy after week 20 (one loss or more) I usually obtain:
- factor V Leiden genetic test
  - prothrombin 20210 mutation genetic test
  - protein C activity
  - protein S activity and total and free protein S antigen
  - antithrombin III activity
  - antiphospholipid antibodies, consisting of:
    - lupus anticoagulant
    - anticardiolipin IgG and IgM antibodies
  - homocysteine level

Important issues when testing or interpreting thrombophilia tests:

- Protein C and S activity are both low in the patient being treated with warfarin (Coumadin), and are therefore not helpful in this situation. A patient needs to have been off warfarin for at least 14 days, before results return to the normal baseline and are reliable. Some patients have been told they have "combined protein C and S deficiency", but on review of their records it becomes clear that they were tested while on Coumadin. Patients with liver disease may have low values of protein C and S activity, because the two proteins are produced in the liver.
- Protein C "activity" levels, not "antigen" levels, should be obtained. The antigen test measures how much of the protein is present, no matter whether it works (= is functional) or not. The activity measures, how much protein works correctly. The patient who has a normal amount of protein C (i.e. normal antigen), but a protein that does not work right (i.e. low activity), has protein C deficiency. Thus, normal antigen levels do not rule out a deficiency. If your lab report only states "protein C", without indicating whether it was activity or antigen was tested, your doctor should clarify which of the two it was. I rarely obtain protein C antigen levels.
- Protein S "activity" levels should optimally be obtained. Since the activity assay is a test that is notoriously difficult to perform and not infrequently gives unreliable results, I always repeat values that were found to be low at labs other than our university lab. Furthermore, since the test is difficult to perform and the results may be unreliable, I also obtain protein S antigen levels. Protein S antigen levels are often called "free protein S" and "total protein S" on the lab test forms (see [Q/A 50](#)). Normal protein S antigen levels do not reliably exclude a protein S deficiency (see discussion above under "protein C"), since a deficiency due to a dysfunctional protein S

may be missed if one only obtains antigen levels. Protein S activity and antigen levels are very often low in the woman on oral contraceptives, on hormone replacement therapy, and during pregnancy. This does not mean that the woman has inherited "Protein S deficiency". Protein S deficiency" should therefore not be diagnosed in these situations

- Antithrombin III (=ATIII) activity can be low in the first few days of an acute DVT or PE, without the patient having true antithrombin III deficiency. Retesting at a later point is necessary. Patients who lose protein in their urine (proteinuria) may have low values, as may patients with liver disease.
- Homocysteine levels can be falsely increased to a mild degree, if the blood was not transported on ice to the lab or not spun within 60 minutes of collection.
- The lupus anticoagulant test can be difficult to perform. When the patient is on heparin the test may be negative, i.e. the patient may still have a lupus anticoagulant (= false negative test); the test may also be false negative if the patient is on warfarin; or if the blood sample was not spun correctly in the lab. The test is not infrequently misinterpreted. The interpreting physician really has to know what type of lupus anticoagulant test kit his/her lab uses and how the lab performs the test.
- Anticardiolipin antibodies: The test is poorly standardized, that is: tests at different laboratories may give different results. The test must be interpreted with caution. The absolute level of the test result is important, i.e. not just whether the test is "positive" or "negative".
- Factor VIII and fibrinogen activity levels can be high in the acute setting of a DVT or PE or at times of acute illness or infections, and should be tested once the acute events are over, such as 3 months later.
- APC resistance (= activated protein C resistance): discussed in [Q/A 31](#).

#### Reference:

1. A useful medical reference article on thrombophilia testing: *Annals of Internal Medicine*. 135(5):367-73, 2001 Sep 4: "The thrombophilias: well-defined risk factors with uncertain therapeutic implications".