DISORDER/SETTING

Question 1: What is the specific clinical disorder to be studied?
Question 2: What are the clinical findings defining this disorder?
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Question 4: What DNA test(s) are associated with this disorder?
Question 5: Are preliminary screening questions employed?
Question 6: Is it a stand-alone test or is it one of a series of tests?
Question 7: If it is part of a series of screening tests, are all tests performed in all instances (parallel) or are only some tests performed on the basis of other results (series)?

DISORDER/SETTING

Question 1: What is the specific clinical disorder to be studied?

The disorder being considered in this review is an episode of recurrent venous thrombosis in adults with an inherited clotting disorder.

The purpose of this ACCE review is to evaluate the efficacy of identifying an inherited clotting tendency after an episode of venous thrombosis and intervening to avoid a second episode. The overriding issue is whether knowing that such individuals carry a disease-causing mutation will allow specific treatment that reduces risk for a second attack, over and above what might be recommended in the absence of such knowledge.

DISORDER/SETTING

Question 2: What are the clinical findings defining this disorder?

The most common presentations of venous thrombosis are deep vein thrombosis of the lower extremity and pulmonary embolism. The causes of venous thrombosis can be divided into two groups: hereditary and acquired. There is often more than one factor at play in a given patient. As an example, 50 percent of thrombotic events in patients with hereditary causes are associated with an acquired risk factor such as surgery, pregnancy, or oral contraceptives. In addition, more than one form attributable to an hereditary or acquired cause can be present in a given patient. The management of venous thrombosis involves avoidance of the multiple risk factors, as well as anticoagulation. The presence of an hereditary cause may affect the management of individuals with venous thrombosis.

Deep Vein Thrombosis

The cardinal symptoms and signs of lower extremity deep vein thrombosis (DVT) are erythema, warmth, pain, swelling, or tenderness of the leg or calf. Tenderness on deep palpation of the calf muscles is the major physical findings. However, many clinical investigations have established that DVT cannot be reliably diagnosed on the basis of the history and physical examination, even in high-risk patients (Wheeler 1985; Lensing, *et al.*, 1989). Patients with lower extremity DVT often do not exhibit erythema, warmth, pain, swelling, or tenderness, as shown in Table 1.

Table 1: Comparison of Multiple Clinical Studies of DVT (Leclerc, *et al.,* 1991)

Symptom or Sign	Sensitivity (Range)	Specificity (Range)
calf pain	66-91%	3-87%
calf tenderness	56-82%	26-74%
Homan's sign	13-48%	39-84%
swelling of calf or leg	35-97%	8-88%

When present, these findings merit further evaluation, despite the poor specificity. Thus, the clinical evaluation may imply the need for further evaluation but cannot, by itself, be relied on to confirm or exclude the diagnosis of DVT.

Pulmonary embolism-

Pulmonary embolism (PE) should be considered whenever unexplained dyspnea occurs, as well as in all patients with DVT. Dyspnea, with or without associated anxiety, as well as pleuritic chest pain and hemoptysis, are common in PE. Tachypnea and tachycardia are the most common signs. However, as with DVT, all of these symptoms and signs are nonspecific. Lightheadedness and syncope may be caused by PE but may also result from a number of other entities that result in hypoxemia or hypotension. Pulmonary embolism should always be suspected in the setting of syncope or sudden hypotension, and these often indicate a large clot

burden. The cardiac and pulmonary physical examinations are both nonspecific for PE. Dyspnea, tachypnea, clear lung fields, and hypoxemia are the most likely findings. Finds on physical examination of the heart and lungs are not specific for PE. When the clinical presentation is consistent with PE, the lack of specificity mandates that additional testing, such as ventilation-perfusion scan or pulmonary angiography be performed.

Prevalence

The prevalence of venous thrombosis and pulmonary embolism is approximately 1 per 1000 individuals per year at age 50-55 increasing with age to about 1 in 100 by age 85 (see Cumulative Incidence Question 21).

DISORDER/SETTING

Question 3: What is the clinical setting in which the test is to be performed?

The setting is *a confirmed recent episode of deep venous thrombosis in adults.* This setting is chosen, because it would be favorable for finding a role for a hereditary disorder. In the present review, clinical evaluation is limited to an event within the past year, because most of the data for assessing efficacy of intervention have been collected within that timeframe.

- *venous thrombosis* refers to an episode of inappropriate obstructive clotting in a vein frequently associated with embolization and resultant morbidity and mortality
- *recent* refers to venous thrombosis occurring within one year
- *confirmed* refers to confirmation by invasive or non-invasive testing. The American Thoracic Society guidelines for confirmation are as follows:
  - Venous thrombosis of the lower extremities is confirmed by a positive compression ultrasonogram, impedance plethysmogram, magnetic resonance venogram or contrast venogram.
  - Venous thrombosis at unusual sites is confirmed by a positive imaging study (e.g. magnetic resonance imaging of the venous sinuses of the central nervous system).
  - Pulmonary embolism is confirmed by a high-probability ventilation-perfusion scan using the PIOPED criteria (PIOPED 1990) or pulmonary angiogram.

The purpose of this review is to determine the extent to which management might be improved in such cases, if an hereditary cause were to be identified. It is now possible to detect two hereditary causes.

Clinical evaluation, including testing, is to be directed at individuals with *a recurrent venous thrombosis event occurring in the past year.*

- *a venous thrombosis event* means a confirmed episode of deep vein thrombosis or pulmonary embolism.

Other settings in which testing for these mutations are being conducted include: asymptomatic individuals with a family history of venous thrombosis or known mutation; asymptomatic individuals undergoing major surgical procedures; asymptomatic individuals before initiation of oral contraceptives or hormone replacement therapy. These settings are not reviewed, because in many of these situations treatment is indicated whether an inherited clotting tendency is present or not.

DISORDER/SETTING

Question 4: What DNA test(s) are associated with this disorder?

factor V Leiden (FVL)
FVL is a designation given to a mutation in factor V, characterized by a single base pair change resulting in an arginine to glutamine substitution. This results in a factor V molecule that is not cleaved by Protein C as effectively as wild type factor V, thereby increasing the tendency to clot. Individuals heterozygous for FVL have a seven-fold increased relative risk for venous thrombosis; homozygous individuals have a relative risk of 80. DNA testing for FVL is available and will be assessed, as part of this review. [For more background on FVL, please see Appendix].

Prothrombin gene 20210A mutation (PRO)
PRO is a designation given to a mutation characterized by a single nucleotide change in the 3' untranslated region of the prothrombin gene. This substitution leads to higher levels of prothrombin and an increased tendency to clot. Individuals heterozygous for the PRO mutation have a relative risk of 2.8 (95% CI 1.4 to 5.6). DNA testing for PRO is available and will also be assessed, as part of this review.

Laboratory Tests
Multiple PCR based methods have been used clinically to detect single mutations to establish the presence of *FVL* or *PRO* (Pecheniuk, *et al.*, 2000). The methods use gel-based or non-gel-based technologies. Gel-based technologies include PCR-restriction length polymorphisms, allele-specific PCR/amplification refractory mutation systems (ARMS™), single-stranded conformational polymorphisms (SSCP) and heteroduplex analysis. Non-gel-based technologies include hybridization with allele-specific oligonucleotides, microparticle enzyme immunoassay, oligonucleotide ligation assays, minisequencing, TaqMan, and molecular beacons.

Equivalent non-DNA test-
The mutation in the factor V molecule was discovered because addition of activated Protein C (APC) to patient sera failed to prolong the modified activated partial thromboplastin time (aPTT) test. The modified aPTT test system contains plasma that is factor V deficient. If APC and the patient's sera fail to result in prolongation of the clotting time, it is reported as positive, meaning the patient has "APC resistance". This is equivalent to finding the factor V Leiden mutation in over 90 percent of cases. However, other causes of APC resistance besides the presence of a heterozygous *FVL* mutation can account for the test being positive, including homozygous *FVL* mutation, other rare *FVL* mutations, or other undefined causes. By measuring a ratio between results from a normalized control and the patient, heterozygotes can be separated from homozygotes. However, the possibility that other causes are present can not be ruled out by the APC resistance test alone. In addition, there are multiple difficulties involving the test system in equating the APC resistance test with the DNA test (Favaloro, *et al.*, 1999).

DISORDER/SETTING

Question 5: Are preliminary screening questions employed?

In the present study, preliminary questions would be directed at determining whether the affected individual's previous thrombotic event was adequately documented. This would involve review of the kinds of diagnostic studies listed in Question 2.

DISORDER/SETTING

Question 6: Is it a stand-alone test or is it one of a series of tests?

DNA Testing: Factor V Leiden and prothrombin G20210A mutation

These are stand-alone tests, although they are often performed in combination as a multiplex PCR.

DISORDER/SETTING

Question 7: If it is part of a series of tests, are all tests performed in all instances (parallel) or are some tests performed only on the basis of other results (series)?

DNA Testing: Factor V Leiden and prothrombin G20210A mutation testing are not performed as part of a series of tests.

References

Bertina RM, Koeleman BP, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, van der Velden PA, Reitsma PH. 1994. Mutation in blood coagulation factor V associated with resistance to activated protein C. *Nature* **369**: 64-67.

Dählback B, Carlsson M, Svensson PJ. 1993. Familial thrombophilia due to a previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C: prediction of a cofactor to activated protein C. *Proc Natl Acad Sci U S A* **90**: 1004-1008.

Favaloro EJ, Mirochnik O, McDonald D. 1999. Functional activated protein C resistance assays: correlation with factor V DNA analysis is better with RVVT-than APTT-based assays. *Br J Biomed Sci* **56**: 23-33.

GeneTests. 2002. GeneTests·GeneClinics: Medical Genetics Information Resource [database online]. Copyright, University of Washington and Children's Health System, Seattle. 1993-2001. Updated weekly.

Koster T, Rosendaal FR, de Ronde H, Briët E, Vandenbroucke JP, Bertina RM. 1993. Venous thrombosis due to poor anticoagulant response to activated protein C: Leiden Thrombophilia Study. *Lancet* **342**: 1503-1506.

Koster T, Rosendaal FR, Briët E, van der Meer FJ, Colly LP, Trienekens PH, Poort SR, Reitsma PH, Vandenbroucke JP. 1995. Protein C deficiency in a controlled series of unselected outpatients: an infrequent but clear risk factor for venous thrombosis (Leiden Thrombophilia Study). *Blood* **85**: 2756-2761.

Leclerc JR, Illescas F, Jarzem P. 1991. Diagnosis of deep vein thrombosis. In: *Venous Thromboembolic Disorders*. Leclerc JR (ed). Lea Febiger: Philadelphia; 176.

Lensing AW, Prandoni P, Brandjes D, Huisman PM, Vigo M, Tomasella G, Krekt J, Wouter Ten Cate J, Huisman MV, Büller HR. 1989. Detection of deep-vein thrombosis by real-time B-mode ultrasonography. *N Engl J Med* **320**: 342-345.

Pecheniuk NM, Walsh TP, Marsh NA. 2000. DNA technology for the detection of common genetic variants that predispose to thrombophilia. *Blood Coagul Fibrinolysis* **11**: 683-700.

PIOPED. 1990. Value of the ventilation/perfusion scan in acute pulmonary embolism. Results of the prospective investigation of pulmonary embolism diagnosis (PIOPED). *Jama* **263**: 2753-2759.

Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. 1996. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. *Blood* **88**: 3698-3703.

Wheeler HB. 1985. Diagnosis of deep vein thrombosis. Review of clinical evaluation and impedance plethysmography. *Am J Surg* **150**: 7-13.

QUESTION 4 APPENDIX

factor V Leiden (*FVL*)

In the late 1980s, it was recognized that some families with an inherited deficiency of protein C did not have an increase in incidence of venous thrombosis – the expected consequence of protein C deficiency. Activated protein C (APC) is a protease with potent anticoagulant properties. During normal hemostasis, APC inactivates factor Va and factor VIIIa by proteolysis, thus inhibiting the clotting cascade. In its absence, a procoagulant effect would be expected. Controversy surrounded this finding and led to the formation of the Leiden Thrombophilia Study (LETS), a large case-control study aimed at investigating risk factors associated with venous thrombosis (Koster *et al.,* 1995). A major result of this study was the discovery of the cause of "APC resistance" – another interesting observation to do with protein C. This phenomenon was first uncovered by a novel test in which an activated partial thromboplastin time is determined before and after APC is added to patient sera (Dählback, *et al.,* 1993). In the LETS, large percentages of patients with a personal or family history of venous thrombosis were found to have APC resistance (Koster, *et al.,* 1993). Interestingly, control populations also had a high percentage of APC resistance (approximately 5 percent). Following linkage analysis that confirmed that APC resistance in affected families was associated with factor V, and the identification of individuals with extreme resistance (later shown to be homozygous for *FVL*), the mutation was identified. This single basepair change (nucleotide position 1691, G to A substitution) resulted in an arginine to glutamine change in factor V at position 506 (FV R506Q, or FV Leiden) (Bertina, *et al.,* 1994). This results in a factor V molecule that is not cleaved by activated protein C as effectively as wildtype factor V. The LETS identified a relative risk of venous thrombosis of 7 for individuals heterozygous for *FVL* and 80 for homozygous individuals.

Prothrombin gene G20210A mutation (*PRO*)

In 1996 an additional procoagulation defect was reported among individuals in the LETS (Poort, *et al.,* 1996). Using a candidate gene approach, the authors looked for mutations in the prothrombin gene. In selected patients with a familial history of venous thrombosis they sequenced the exons and the 5'- and 3'-UT region of the prothrombin gene. A single nucleotide change (G to A at position 20210) was identified in the sequence of the 3'-UT region. Eighteen percent of the patients had the G20210A genotype, as compared with 1% of controls. In a population-based case-control study, the G20210A allele was identified at a frequency of 1.2 percent in controls and 6 percent in unselected patients. The presence of the mutation increased the risk of venous thrombosis almost threefold (odds ratio, 2.8; 95 percent confidence interval, 1.4 to 5.6). The authors found the G20210A allele was associated with elevated prothrombin levels. This was thought to be the mechanism for increased thrombosis associated with the mutation since elevated prothrombin itself also was found to be a risk factor for venous thrombosis.