Factor V Leiden with Deep Venous Thrombosis

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Factor V Leiden (FVL) is an autosomal co-dominantly inherited Arg^{506}→Gly substitution of the activated protein C cleavage site affecting 5% of the Caucasian population. FVL results in impaired anticoagulant function without procoagulant modification. Heterozygotes experience a seven-fold increase in thrombotic events, whereas homozygotes may incur a 50 to 100 fold increase. Even though patients are at increased risk for deep venous thrombi, they experience a smaller risk of pulmonary embolism compared to individuals affected by other coagulopathies.

ABBREVIATIONS: APC = activated Protein C; APTT = activated partial thromboplastin time; CT - computed tomography; DVT = deep venous thrombosis; FV = factor V; FVL = Factor V Leiden; INR = international normalized ratio; LAC = lupus anticoagulant; PE = pulmonary embolus; PNH = paroxysmal nocturnal hemoglobinuria; PT = prothrombin time; RBC = red blood cell; SLE = systemic lupus rythematosus; V/Q ratio = ventilation/perfusion ratio.

INDEX TERMS: Factor V Leiden; Protein C; thrombosis.

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PRESENTATION OF PATIENT

A 45-year-old white female presented to an emergency room complaining of fever, cough, and left leg pain. Duplex ultrasonography revealed a left greater saphenous thrombus with extension into the left common and femoral veins. The patient was discharged on oral warfarin. Two days later the patient returned to the hospital complaining of abdominal swelling, left upper quadrant pain, and epigastric tenderness. Additionally, the prothrombin time (PT) performed upon admission yielded an international normalized ratio (INR) of 1.0. A computed tomography (CT) scan revealed bilateral lung effusions without atelectasis and splenomegaly with multiple low density splenic masses. The patient stated that she experienced slight dyspnea upon exertion. The ventilation perfusion scan was not diagnostic for pulmonary embolus (PE). Upon interview, the patient revealed that she had two prior deep venous thrombi (DVT). Her first occurred at age 25 in her left leg after delivery of her second child. The second DVT occurred at age 43 in her right leg. Also of interest is the death of her daughter subsequent to a massive PE at age 22. Due to a suspected familial coagulopathy, the patient was started on IV heparin and referred to a tertiary care hospital for treatment and follow up.

Given her current illness and past medical history, she was evaluated for paroxysmal nocturnal hemoglobinuria (PNH) vs. familial coagulopathy vs. lupus anticoagulant (LAC). Oral warfarin therapy was initiated concurrent with IV heparin until her INR was therapeutic. Laboratory values are shown in Tables 1 and 2.

QUESTIONS TO BE CONSIDERED

1. What is the most important 'clue' when working-up a patient with a suspected thrombophilia?
2. What laboratory testing would be most appropriate to rule out each of the above differential diagnoses?
3. What mechanisms are responsible for the homeostatic imbalance with Factor V Leiden?
4. What laboratory testing would be most beneficial in the diagnosis of this patient?
5. Given the patient's diagnosis and history, what would be the preferred course of treatment and why?

NON-LABORATORY DIAGNOSIS OF DVT AND PE

In conjunction with a good patient history and physical exam, the diagnosis of DVT is frequently made using non-invasive ultrasonography. Ultrasound may be used to directly visualize

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the thrombus or the Doppler effect may be utilized to measure blood velocity. The diagnosis of PE is generally made via assessing the ventilation/perfusion ratio (V/Q ratio). Isotope labeled xenon gas is inhaled into the lungs and held. A gamma camera is then used to visualize all ventilated areas. The patient is then injected with isotope labeled albumin. Due to the embolic impedance of blood flow, the radioactive albumin is concentrated within the pulmonary capillary beds. This concentration allows for an indirect measure of blood flow. The normal result of a V/Q scan is 1. If V is greater than Q, the quotient is greater than 1 and thus supports the diagnosis of PE.[1]

PNH and LAC

PNH is an acquired genetic mutation of hematopoietic stem cells resulting in an increased sensitivity of daughter cells to the actions of complement. Although the lysis of red blood cells (RBCs) and resultant nocturnal hemoglobinuria were first identified, patients more commonly suffer from recurrent venous thromboembolism of the abdominal circulation. As such, PNH becomes a differential diagnosis in any patient with abdominal thrombi.[2]

LAC refers to one of two types of antiphospholipid antibodies. As implied by its name, this inhibitor was originally found to be the cause of elevated activated partial thromboplastin time (APTT) in patients with systemic lupus erythematosus (SLE). In contrast to this in vitro observation, patients often experience a hypercoagulable state concurrent with venous and arterial thrombi.[3]

Table 1. Laboratory results

Test	Result	Reference range
Complete blood count (CBC)	unremarkable	
Antiphospholipid antibodies	within normal limits	
Sucrose hemolysis	negative	
Anti native DNA	2.3 IU/mL	0 – 3 IU/mL
ANA	< 1:40	<1:40
Homocystine	7 µmol/L	5 – 8 µmol/L
20210 Downstream PT gene mutation	negative	
Factor V Leiden mutation	homozygous positive	

Table 2. PT and APTT results

Date	PT/INR (10.1-13.7 sec)	APTT (25.8 - 39.4 sec)
12/8	16.0 sec/1.3	105.0
12/9	16.6 sec/1.3	96.7
12/10	21.1 sec/1.7	101.4
12/11	28.8 sec/2.2	148.9

FACTOR V AND ITS ROLE IN NORMAL HOMEOSTASIS

Factor V (FV), like thrombin, possesses both anticoagulant and procoagulant properties. The activated protein C (APC) mediated cleavages, if performed on FV (not FVa), transform it into an APC co-factor (FVac). FVac acts in concert with APC and protein S to increase the rate of inactivation of FVIII.

FV may also be cleaved by thrombin into FVa, a procoagulant. This molecule acts as a co-factor and combines with FX to form the prothrombinase complex. Conversely, FVa may be inactivated by APC cleavages resulting in a form without anticoagulant activity. More specifically, FVa is cleaved by APC at Arg 306, Arg 506, and Arg 679, after binding to phospholipid on the endothelial surface. The Arg 506 cleavage has little *direct* affect on the procoagulant activity of the FV molecule. In a recent study, FV activity was assayed before and after cleavage at Arg 506 and 679. These cleavages conferred only a 20% reduction in activity.[4] Expectedly, the Arg 306 cleavage has been found responsible for an 80% reduction in FV activity. The primary function of the Arg 506 cleavage on FV is to augment the *rate* of inactivation via the Arg 306 cleavage.

WHAT IS THE LEIDEN MUTATION AND HOW DOES IT AFFECT FV?

The FV gene is located on the long arm of chromosome 1 between bands 21 and 25 and is autosomal co-dominantly inherited.[4] Factor V Leiden (FVL) results from a G→A point mutation at position 1691 resulting in an Arg^{506}→Gly substitution in the protein.[5] This substitution renders FV resistant to the activity of APC. The net effect of this mutation predominantly

alters the anticoagulant properties of FV while leaving the procoagulative function intact.

The clinical presentation of FVL ranges from asymptomatic to serial DVTs and other venous infarcts. The severity of the disease is proportional to the number of additional risk factors present in conjunction with the Leiden mutation.[7] In general, risk factors for DVT are pregnancy, hormonal birth control, venous stasis, surgery, obesity, smoking, immobilization, cancer, homocysteinemia, and other familial coagulopathies.[1] Consequently, the presence of FVL alone may not be sufficient to precipitate an event. However, FVL in conjunction with any number of the aforementioned conditions may cause the homeostatic balance to tip in favor of clot formation.[6,7]

FVL possesses several interesting points of difference from other familial coagulopathies. First, the risk of DVT occurrence is linear throughout the lifetime of the patient.[7] Second, even though patients are at an overall increased risk for thrombi, they face a decreased chance of suffering PE as compared to those with other familial coagulopathies.[8] This paradoxical relationship of increased DVT risk with a decreased frequency of PE exists due to 1) FV's dual role as an anti and procoagulant, and 2) the anatomic location in which FVL patients frequently form clots. First, both *wt*-FV and *wt*-FVa are cleaved by APC at Arg 306, 506, and 679. The 506 cleavage in FV transforms it into an anticoagulant APC cofactor for the degradation of FVIII. Unbound FVa also depends upon the 506 cleavage to increase the rate of its molecular inactivation.[9] This duality is a crucial component of the FVL paradox. Cleavage at the Arg 506 site is only necessary for anticoagulant functions. The $Arg^{506} \rightarrow Gly$ mutation destroys this cleavage site thus decreasing the rate of free FVa inactivation and decreasing the formation of FVac on the surrounding undamaged endothelium. Due to increased thrombin generation and decreased anticoagulant activity on surrounding tissue, an adherent clot is rapidly formed that results in decreased embolization.

The second component of the Leiden paradox arises from the fact that PE typically arise from thrombi in the larger proximal veins such as the ileo-femoral and inferior vena cavum.[9] In contrast, FVL patients incur thrombi with a greater frequency in the distal calf veins (anterior tibial, posterior tibial, and peroneal veins).[10,11] This location decreases the probability embolus formation.[12]

DETECTION, DIAGNOSIS, AND TREATMENT

In the initial assessment of a patient with DVT, history is often key to the detection of familial clotting disorders. As demonstrated by our patient, the occurrence of DVT in family members or a history of DVT can be an invaluable diagnostic tool. Typically once a FVL mutation is suspected, the APC resistance test is performed. The APC resistance test identifies patient insensitivity to APC. The test is based upon an APTT clotting assay with and without reagent APC. The APTT in the presence of APC is divided by the unaltered APTT to yield a unit less ratio. A ratio of >2 generally confers an unaffected state. Ratios of <2 denote a potential FVL mutation and resistance to APC. FV deficient plasma may be added to the test system to correct for any existing factor deficiencies. The APC resistance assay may be affected by other conditions such as LAC, elevated FVIII and fibrinogen levels, pregnancy (elevated acute phase reactants, e.g., FVIII, C4b-binding protein), and hormonal contraceptives.[13,14]

APC resistant patients may be confirmed for the FVL mutation by DNA PCR amplification of a segment of the potentially affected gene. After amplification, the DNA is cut with a restriction enzyme and run through a polyacrylamide gel. The DNA fragments separate on the basis of size allowing identification of the individual gene fragments. Homozygous negative patients have three bands (82, 37, and 104 bp), heterozygous have four bands (82, 37, 104, and 141 bp), and homozygous positive have two bands (82 and 141 bp).[15]

As with the above patient, testing may be done not only to confirm APC resistance and the FVL mutation, but also to rule out other potential interfering problems and manage the anticoagulant therapy. Both PNH and LAC are associated with thrombi and thus were differential diagnoses. PNH, a polyclonal hematopoietic stem cell disorder, produces progeny that lack GPI anchored proteins.[2] Most important to our discussion is the absence of decay accelerating factor (DAF aka CD55) and membrane inhibitor of reactive lysis (MIRL aka CD 59) on daughter RBCs. The absence of these proteins renders these cells incapable of mitigating the actions of the alternative complement pathway. As such, the abnormal susceptibility to complement mediated lysis is utilized as a screening tool within the sucrose hemolysis and Ham's acidified serum tests. As presented in Table 1, the CBC was unremarkable and did not demonstrate anemia. Red blood cell susceptibility to complement was ruled out via a negative sucrose hemolysis test thus precluding a diagnosis of PNH.[16]

SLE was eliminated since the ANA, a nonspecific indicator of many connective tissue diseases, was within normal limits as was the anti ds-DNA, a more specific indicator of SLE. Antiphospholipid antibodies, often associated with SLE and thrombotic disease, were found to be within normal limits and thus precluded LAC.[17]Additionally, the patient had a normal homocystine level and was negative for the PT gene mutation. Most important, our patient was homozygous positive for the FVL mutation. The patient was started on oral warfarin and intravenous heparin concurrently. After the PT reached therapeutic levels, an INR between 2 and 3, the heparin was discontinued. Taking into account her thrombotic history and the aforementioned homozygous FVL mutation, the patient was asked to continue on oral warfarin for life and to return for follow-up in three months.

REFERENCES

1. Thompson BH, Stanford W. New approaches in imaging pulmonary embolism. In: Fauci AS, Braunwauld E, Isselbacher KJ, and others, editors. Harrison's principles of internal medicine. New York: McGraw-Hill; 1998. p 1203-60.
2. Lee GR. Hemolytic disorders: Wintrobe's clinical hematology. Baltimore: Lippincott Williams and Wilkins; 1999. p 1267.
3. Ravel R. Blood coagulation: Clinical laboratory medicine. Baltimore: Mosby; 1995. p 102.
4. Kalafatis M, Mann KG. Factor V Leiden and thrombophilia: Arterioscler Thromb Biol 1997;17:620-7.
5. Dahlbäck B. Blood coagulation. Lancet 2000;355:1627-32.
6. Fritsma G. Thrombosis risk. In: Rodak BF, editor. Diagnostic hematology. Philadelphia: WB Saunders; 2001: in press.
7. Price DT, Ridker PM. Factor V Leiden Mutation and the risks for thromboembolic disease: a clinical perspective. Ann Intern Med [serial online] 1997;127:895-903. http://www.acponline.org/journals/annals/15nov97/factorvl.htm. Accessed April 16, 2001.
8. Bounameaux H. Factor V Leiden paradox: risk of deep-vein thrombosis but not of pulmonary embolism. Lance. 2000;356:182-3.
9. Dahlbäck B. Procoagulant and anticoagulant properties of coagulation factor V: factor V Leiden causes hypercoagulability by dual mechanisms: J Lab Clin Med 1999;133:415-22.
10. Leroyer C, Mercier B, Escoffre M, and others. Factor V Leiden prevalence in venous thromboembolism patients: Chest 1997;111:1603-6.
12. Björgell O, Nilsson PE, Nilsson JA, and others. Location and extent of deep vein thrombosis in patients with and without FV:R506Q mutation. Thromb Haemost 2000;83:648-51.
13. Handlin RI. Bleeding and thrombosis: Harrison's online. http://www.harrisonsonline.com/server-java/Arknoid/harrisons/1096-7133/Chapters/ch-060?Page=5&Sect=Sect6. Accessed May 8, 2001.
14. BIOCLOT, aPC sensitivity [package insert]. Ventura, CA: Biopool, International; Rev 06/7/99.
15. Miller, K. Molecular diagnostics. In: Rodak BF, editor. Diagnostic hematology. Philadelphia: WB Saunders; 2001: in press.
16. Payne M, Bell A, Hamby S. Anemias caused by increased destruction of erythrocytes. In: Rodak BF, editor. Diagnostic hematology. Philadelphia: WB Saunders; 1995;203-50.
17. Hahan B. Systemic lupus erythematosus: Harrison's online. http://www.harrisonsonline.com/server-java/Arknoid/harrisons/1096-7133/Chapters/ch-312?Page=1. Accessed May 8, 2001.