TRANSFUSION MEDICINE UPDATE

Institute For Transfusion Medicine January, 2001

ACTIVATED PROTEIN C RESISTANCE: AN UPDATE

Andrea Cortese Hassett, Ph.D., Scientific Director, Coagulation Laboratory
Franklin A. Bontempo, M.D., Medical Director, Coagulation Laboratory

INTRODUCTION

The phenomenon of activated protein C resistance (APCr) was first reported by Dahlback (1) et al. in 1993 and refers to the inability to mount an effective anticoagulant response. Clinically this results in an increased risk of thrombosis. Most cases of APC resistance are associated with a single point mutation in the factor V gene (Leiden mutation), which results in the substitution of arginine at position 506 by glutamine. Cleavage of this site by APC is necessary for exposure of the two additional cleavage sites needed for inactivation. The rate of inactivation of factor V Leiden (FVL) is therefore slower than that of normal factor V. *In vivo* this manifests as an 8 fold increased risk of thrombosis in heterozygotes and a 50 to 100 fold increased risk of thrombosis in homozygotes (2,3).

LABORATORY METHODOLOGY

The presence of the FVL allele is the major cause of APCr and a well-documented risk factor for venous thrombosis. APC resistance testing is performed on citrated plasma (blue tops) preferably before the patient is on anticoagulants. The first generation, original assay consists of a standard APTT test performed in both the absence and presence of commercially available activated protein C. In a normal patient, the APTT is prolonged in the presence of APC due to the anticoagulant action of this protein. Samples from patients with FVL will fail to prolong the clotting time in the presence of APC resulting in "resistance to APC". The results are reported as a ratio of the APC-APTT/APTT. Affected patients will have an abnormally low ratio compared to controls, usually less than 2.0; levels elevated above normal currently have no known clinical significance.

A second generation, modification of the original assay is currently the method of choice. Patient plasma is diluted with factor V-deficient plasma. This limits variations in plasma handling, minimizes the effects of oral anticoagulant therapy, and improves the sensitivity and specificity for the Leiden allele to nearly 100 percent, while making it cost efficient. If a patient has a normal result with this assay, one does not need to proceed with more costly DNA testing for the Leiden mutation.

CLINICAL MANIFESTATIONS

The penetrance of clinical symptoms is highly variable in APC resistant individuals. Some never get thrombosis, whereas others suffer from recurrent severe thrombotic events. Approximately 35% of APCr individuals experience a thrombotic event at some time in their lives. Carriers also have an increased risk of miscarriage that can present as preeclampsia, placental abruption, intrauterine growth retardation, or stillbirth. Homozygosity for the Leiden mutation gives a much higher risk of thrombosis than heterozygosity. The risk of thrombosis is increased by the coexistence of other genetic or acquired risk factors (4). Other genetic risk factors for thrombosis include deficiencies of protein C, protein S, or antithrombin III, mutations in the genes for factor II (prothrombin) and the homocysteine-related, methylene tetrahydrafolate reductase (MTHFR). The most common acquired risk factors are the use of oral contraceptives, pregnancy, trauma, surgery and the presence of antiphospholipid antibodies (lupus anticoagulants). It is therefore important to consider all of these factors when determining the true risk of thrombosis in a given patient with a positive APCr test.

GUIDELINES FOR TESTING

Guidelines for APC resistance and/or the FVL allele testing include the following clinical situations: 1) venous thromboembolic disease before 50 years of age, 2) family history of venous thrombosis, 3) recurrent venous thrombotic disease, 4) venous thrombosis involving unusual sites, 5) arterial thrombosis in the young (<18 years), 6) patients known to carry other prothrombotic genetic defects, and 7) first-degree relatives (usually >10 yrs) of thrombotic patients with APC resistance.

PATIENT MANAGEMENT

Patients with first time venous thrombosis and FVL mutation who resolve with standard courses of anticoagulant therapy and have no other thrombotic risk factors usually can have anticoagulant therapy stopped. Should a second venous thrombosis occur, indefinite anticoagulant therapy is recommended. Patients with venous thrombosis, FVL and a second congenital or acquired risk factor usually require indefinite anticoagulation. Asymptomatic carriers of FVL must be prophylactically anticoagulated for surgical procedures. Anticoagulation for an asymptomatic pregnant carrier is currently not recommended. As always, patient treatment regiments may need to be individualized.

FUTURE CONSIDERATIONS

The factor V Leiden mutation accounts for approximately 90% of patients with APCr. Recent investigations have centered on the elucidation of other causes of APCr. At least two other genetic mutations, which contribute to an increased risk of thrombosis, have been identified, both of which may lead to an abnormal test for APCr (R2 allele and FV Cambridge; (5-6)). In addition, studies are currently underway to determine whether a reduced sensitivity for APC, not due to a genetic mutation, is an independent risk factor for venous thrombosis (7). Ongoing investigation into these other causes of APCr will lead to an increased understanding of yet more mechanisms underlying thromboembolic disease.

REFERENCES

1. Dahlback B., et. al. 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. 1993: 90: 1004-1008.

2. Rosendaal FR., et. al. High risk of thrombosis in patients homozygous for factor V Leiden (activated protein C resistance). Blood 1995; 85: 1504-1508.

3. Ridker PM., et. al. Mutation in the gene coding for coagulation factor V and the risk of myocardial infarction, stroke, and venous thrombosis in apparently healthy men. N Engl. J. Med. 1995; 332: 912-917.

4. Zoller B., et. al. Identification of the same factor V gene mutation in 47 out of 50 thrombosis-prone families with inherited resistance to activated protein C. J. Clin. Invest. 1994; 94: 2521-2524.

5. Bernardi, EM., et. al. A factor V genetic component differing from factor V R506Q contributes to the activated protein C resistance phenotype. Blood 1997; 90: 1552-1557.

6. Williamson, D., et. al. Factor V Cambridge: A new mutation (Arg^{306}→Thr) associated with resistance to activated protein C. Blood 1998; 91: 1140-1144.

7. deBisser, MCH., et. al., A reduced sensitivity for activated protein C in the absence of factor V Leiden increases the risk of venous thrombosis. Blood 1999; 93: 1271-1276.

Copyright ©2001, Institute For Transfusion Medicine

Any questions concerning,
please contact Andrea Cortese Hassett, Ph.D. or
Franklin A. Bontempo, M.D. at 800-967-9672.
or by e-mail:acortese_hassett@itxm.org
or fbontempo@itxm.org
Copies of the *Transfusion Medicine Update* can be obtained by calling Deborah Small at (412) 209-7320; or by e-mail: dsmall@itxm.org

TMU ADDENDUM: Nov/Dec,2000 Issue
Hemoglobin Based Red Cell Substitutes

In last month's TMU on blood substitutes, we inadvertently omitted mention of PolyHeme®, the hemoglobin solution produced by Northfield Laboratories (Evanston, Illinois; 847-864-3500). This solution contains human hemoglobin that has been cross-linked using pyridoxal phosphate, and then polymerized using glutaraldehyde. The product has a P50 that is slightly higher than that of red cell hemoglobin, and an intravascular half-life of approximately 24 hours. Two clinical trials using PolyHeme® in patients sustaining acute trauma or requiring emergent surgery have been published. The product was well tolerated and was an effective replacement for blood on almost a unit per unit basis in comarable patients, immediately following trauma, as well as for the first 12 hours after surgery. Unlike studies with other hemoglobin solutions, PolyHeme® administration does not result in vasopressor effects. Phase III clinical trials are ongoing. The product is available on a compassionate use basis.

1. Gould SA, Moore EE, Moore FA, et al. J Trauma 1997; 43: 325-32.
2. Gould SA, Moore EE, Hoyt DB, et al. J Am Coll Surg 1998; 187: 113-22.