Prevalence of factor V Leiden in a population of patients with congenital heart disease

Biauw-Chi Ong M MED (Anaesth),*
A. Andrew Zimmerman MD,*
David C. Zappulla BA,†
Ellis J. Neufeld MD PhD,†
Frederick A. Burrows MD*

Purpose: The incidence of thrombotic events following cardiopulmonary bypass (CPB) in patients receiving surgical repair or palliation of congenital heart defects (CHD) is as high as 16%. Protein C, an intrinsic anticoagulation protease which, when activated, breaks down factor Va of the coagulation system, aids in maintaining a normal procoagulant/anticoagulant balance. Resistance of factor Va to degradation by activated protein C occurs and predisposes to thrombotic events. The resistance of factor Va to such degradation is, in the majority of cases, due to a genetic mutation referred to as factor V Leiden (FV_{Leiden}). The presence of FV_{Leiden} can be diagnosed using a DNA based assay. The prevalence of FV_{Leiden} in the with CHD has not been determined. The objective of this study was to determine the prevalence of FV_{Leiden} in patients with CHD.

Methods: Two hundred consecutive patients with CHD undergoing surgical repair or palliation requiring cardiopulmonary bypass were studied. Blood was taken before administration of homologous blood transfusion and assayed using a DNA based method with polymerase chain reaction amplification for the FV_{Leiden} mutation.

Results: The prevalence of FV_{Leiden} in our study population was 9/200 (4.5%). None of these patients demonstrated thrombotic complications. However, three patients (1.5%) without the FV_{Leiden} mutation developed postoperative thrombotic complications.

Conclusions: The prevalence of FV_{Leiden} in patients is 4.5% that is not different from that of the population at large. There was no identifiable association with the occurrence of postoperative thrombotic events.

Objectif : L'incidence de complications thrombotiques survenant à la suite d'une circulation extracorporelle (CEC) chez des patients qui subissent une chirurgie réparatrice ou palliative d'une malformation cardiaque congénitale (MCC) sont aussi élevés que 16 %. La protéine C, une protéase intrinsèque d'anticoagulation qui, une fois activée, inhibe le facteur Va du système de coagulation, aide à maintenir un équilibre normal entre les effets procoagulant et anticoagulant. La résistance du facteur Va à la dégradation par la protéine C activée se produisant, elle prédispose à des accidents thrombotiques. La résistance du facteur Va à une telle dégradation est, dans la majorité des cas, causée par une mutation génétique appelée facteur F Leiden (FV_{Leiden}). La présence du FV_{Leiden} peut être prouvée par une analyse à base d'ADN. La prévalence du FV_{Leiden} n'a pas été déterminée chez les patients qui présentent une MCC et notre étude s'est donc fixé comme objectif de le faire.

Méthode : On a étudié deux cents patients successifs souffrant de MCC devant subir une chirurgie réparatrice ou palliative qui nécessite une circulation extracorporelle. On a prélevé du sang avant la transfusion homologue et on l'a analysé selon une méthode à base d'ADN, utilisant l'amplification en chaîne par polymérase, pour identifier la mutation du FV_{Leiden}.

Résultats : Dans notre étude de population, la prévalence du FV_{Leiden} était de 9/200 (4,5 %). Aucun des patients ne présentait de complications thrombotiques. Toutefois, trois patients (1,5 %) chez qui on n'a pas découvert de mutation du FV_{Leiden}, ont développé des complications thrombotiques postopératoires.

Conclusion : La prévalence du FV_{Leiden} chez les patients de notre étude est de 4,5 %, ce qui n'est pas différent de la prévalence qu'on retrouve dans la population générale. On n'a pu associer l'occurrence d'incidents thrombotiques postopératoires à quelque cause identifiable.

From the Departments of Anaesthesia* and Pediatrics,† Children Hospital and Harvard Medical School, Boston, Massachusetts 02115.
Address correspondence to: Dr. Frederick A. Burrows, Department of Anaesthesia, Slot #203, Arkansas Children's Hospital, 800 Marshall Street, Little Rock, Arkansas 72202 USA. Phone: 501-320-2933; Fax: 501-320-2939
Accepted for publication September 26, 1998

THE blood coagulation system is regulated by procoagulant and anticoagulant proteins in plasma and on the surface of endothelial cells. Under normal physiological conditions, procoagulant and anticoagulant mechanisms are delicately balanced: disturbances in this balance may result in bleeding or thrombotic events. Patients with congenital heart disease (CHD) undergoing surgery with the use of cardiopulmonary bypass (CPB) are at an increased risk for developing coagulation disorders.[1,2]

Venous thrombosis is a potentially devastating complication of cardiac surgery and has been reported to occur in 5% to 16% of children following reparative surgery.[1,3,4] This incidence approximates the prevalence of resistance to activated protein C (APC) in the population at large and a high incidence of anticoagulant protein abnormalities has been found in association with venous thrombotic events in children.[5–7]

Protein C, a vitamin K dependent zymogen of a serine protease, is activated by the thrombin-thrombomodulin complex (thrombin-TM). Thrombomodulin is an endothelial cell product, the expression of which is activated by various stimuli including low blood flow and hypoxia. Circulating thrombin binds to TM producing a thrombin-TM complex. In the presence of the co-factor protein S, APC exerts a potent anticoagulant effect by selectively degrading the procoagulant factors Va and VIIIa. Resistance to the action of APC by factor Va has been described only recently. In patients with resistance to APC the normal plasma response to the anticoagulant activity of APC is reduced.[8] The vast majority of cases (>95%) of resistance of factor Va to degradation by APC is genetically inherited as an autosomal dominant gene, the factor V mutation [R506Q] more commonly referred to as factor V Leiden mutation (FV_{Leiden}). The abnormality is a point mutation in the factor V gene (chromosome 1 q23) at nucleotide position 1691 with guanine substituting for adenine. This results in substitution of arginine by glutamine at position 506 of factor V. Activated protein C inactivates FV_{Leiden} slowly, producing a procoagulant state.[9]

The prevalence of FV_{Leiden} in the population at large is reported to be 5-7%. In patients with demonstrated venous thrombosis the prevalence of FV_{Leiden} has been reported to be 8-64%.[8,10]

Patients with congenital heart disease (CHD) have a higher incidence of chromosomal abnormalities than the population at large.[11] The prevalence of FV_{Leiden} in young patients with (CHD) has never been investigated. Jahangiri *et al.* reported a thromboembolic rate of 10 of 64 patients (15.6%) after the Fontan procedure and in a further evaluation of study of 20 patients having undergone Fontan's palliation the plasma levels of protein C, protein S and factor VII were found to be reduced when compared with the normal range. However, evidence of abnormalities in functional activity was not sought.[4]

The purpose of this study was to determine the prevalence of FV_{Leiden} in a population of patients with congenital heart disease.

Methods

Patients

After approval from the institution's Committee on Clinical Research, 200 consecutive patients with congenital heart disease were studied. Entry criteria included: (1) cardiac surgery requiring the use of cardiopulmonary bypass (CPB); (2) no homologous blood transfusions within two months as a conservative cutoff to avoid possible contamination with donor blood; (3) informed consent. Two milliliters of blood were collected from each patient through the arterial or venous line that were placed for the surgery. The blood was stored at -80°C and transferred to the laboratory for analysis.

Assay

DNA was extracted from frozen blood, and a polymerase chain reaction method known as "amplification refractory mutation system (ARMS)" was used for diagnostic testing, as described by Yandava *et al.*[12] In this method, primer pairs specific for the mutant and normal alleles are used in the mutant primer. Patients with the homozygous mutant gene would fail to amplify with normal primers. Heterozygote samples are amplified with both primers. Water controls and known standards for normal and mutant alleles are run in parallel with each batch of samples. The ARMS assay is 100% specific and sensitive for the genetic anomaly. Concordance of the ARMS assay with biochemical assays for resistance to APC has been determined to be > 95% but < 100% indicating non-[R506Q] mechanisms for resistance to APC exist.[12]

Patient follow-up

Each patient's hospital course was reviewed at a weekly mortality and morbidity conference where evidence of thrombotic episodes were specifically sought. This conference includes representatives of the medical, surgical and nursing staff of the Cardiovascular Intensive Care Unit. Subsequent to patient discharge a chart review was performed to identify later occurring thrombotic episodes. Nursing and physician progress notes and treatment plans were examined for any evidence of venous thrombosis. Superficial peripheral venous thrombosis suspected to be related to pre-

vious *iv* catheter placement were excluded from analysis. Chart reviewers were unaware of the FV_{Leiden} status of the patient at the time of record review.

Results

Patient distribution

Two hundred patients were studied. One hundred and one were male and 99 were female. One hundred and two were 0-1 yr of age, 40 were 1-5 yr, 24 were 5-10 yr and 34 were >10 yr of age. The mean age was 4.6 yr ± 0.55 and the mean weight was 16.2 kg ± 1.4.

The cardiac diagnosis included 52 patients with atrial or ventricular septal defects, 26 with double outlet right ventricle or double inlet left ventricle, 9 with hypoplastic left heart syndrome or single ventricle, 19 with valve related procedures, 38 with tetralogy of Fallot, 21 with transposition of the great arteries, 1 with total anomalous pulmonary venous drainage, 17 with atrio-ventricular septal defects, 6 with truncus arteriosus and 11 with other CHD.

Prevalence of factor V Leiden

The prevalence of FV_{Leiden} in our population of patients with congenital heart disease was 4.5% (nine patients). All diagnosed patients were heterozygous.

Thrombotic complications

The incidence of postoperative thrombotic complications was determined to be 1.5% (3 of 200 patients). These patients demonstrated neither FV_{Leiden}, as determined by genetic assay, nor other identifiable coagulation defect.

The first patient had undergone a fenestrated Fontan palliation. The fenestration thrombosed in the immediate postoperative period necessitating re-opening of the fenestration via cardiac catheterization. The second patient developed an atrial thrombus after the stage 1 repair for hypoplastic left heart syndrome and the third patient developed superior vena caval thrombosis after a Fontan's procedure necessitating revision.

Five patients had a history of thrombotic events. One developed superior vena cava thrombosis secondary to a central venous pressure line and presented for surgical thrombectomy and closure of an atrial and ventricular septal defect. Another patient developed thrombosis of a Fontan palliation that was converted into a bidirectional Glenn shunt. Subsequently this patient underwent a second Fontan palliation and, this time, the postoperative course was unremarkable. Two patients had blocked Blalock-Taussig shunts previously. The last patient had acute thrombosis of a unifocalized pulmonary collateral vessel. The patient was maintained on heparin postoperatively but clot resolution failed to occur and oxygen saturation remained low. He presented for a second procedure that utilized cardiopulmonary bypass, and the postoperative course was unremarkable. He was subsequently diagnosed as a heterozygote for FV_{Leiden}. One patient was known to have protein C deficiency and another to have factor VII deficiency. These two patients were anticoagulated prophylactically. There was no problem with bleeding or thrombosis in the postoperative period.

There were seven deaths in our series of which six were in the 0-1 mo age group, all were due to low cardiac output states and none were associated with thrombotic events.

Discussion

Both quantitative and qualitative abnormalities of coagulation factors and co-factors can render a patient susceptible to the development of thrombosis and thromboembolism. Deficiencies of antithrombin III, protein C and/or protein S were detected in 8-15% of patients who developed thrombosis where an aetiology could be identified.[5] The prevalence of inherited protein C *deficiency* has been reported as 1.45 per 1000 (0.145%) in the population at large and 2-8% in the thrombophilic population.[13] Therefore, the qualitative abnormality of FV_{Leiden} with a prevalence of 5-7% in the population at large and 8-64% in the thrombophilic population is considerably higher than that of inherited protein C *deficiency*.[5,6,10]

Coagulation abnormalities that occur in children undergoing paediatric cardiac operations represent a complex interrelation between coagulation factors and co-factors and may include preexisting coagulation defects as well as defects secondary to CPB.[14,15] Contributing factors towards the thrombotic state in patients undergoing repair of CHD requiring CPB include consumption and dilution of various anticoagulant factors with the institution of cardiopulmonary bypass.[14–16] Such a thrombotic state increases the propensity for the development of thrombosis, particularly in regions of lower flow and/or pressure such as the venous system, and in areas of turbulent flow or stasis, as may occur around vascular anastomosis. Thrombosis in these patients can be as devastating as postoperative bleeding. Two patients in our series; one who developed thrombosis of the Fontan fenestration and the other who developed thrombosis of a Blalock-Taussig shunt, required urgent intervention.

Protein C *deficiency* in children is associated with a higher incidence of clinically significant thrombotic episodes when compared with adults. Additionally, neonates and infants have both reduced plasma levels of protein C as well as reduced functional activity on a

maturational basis. The activity:antigen ratio was reported at 0.67 for newborns compared with 1.0 for adults.[7,17] Theoretically these combined quantitative and qualitative differences should increase the risk of thrombus formation even in the presence of a normal factor V genome. In our study population, 35 patients (17.5%) were less than one month old. Of these 35 patients, one neonate (2.8%) was diagnosed as heterzygous for FV_{Leiden} and one neonate (normal genome) developed a detectable thrombotic episode (superior vena cava thrombus after a Fontan procedure). The neonate with FV_{Leiden} should be at higher risk of thrombotic event post-CPB because of: 1) reduced plasma levels of protein C (maturational etiology and dilutional effect of CPB); 2) reduced functional activity of portein C (maturational etiology) and; 3) the presence of FV_{Leiden}. However, in spite of the presence of these risk factors, the patient's postoperative course was uncomplicated.

The prevalence of FV_{Leiden} was determined to be 4.5% in patients with CHD which was not different from the population at large. The patients diagnosed with FV_{Leiden} did not demonstrate thrombotic events suggesting other factors are necessary to result in thrombosis.

This study suffers from limitations related to the number of patients. The sample size was insufficient to evaluate any relationship between the presence of FV_{Leiden} and postoperative thrombosis. We have calculated that, to determine if FV_{Leiden} (4.5% of patients with CHD) is associated with thrombotic episodes, (1.5% incidence in our study) it would have required approximately 1000 additional patients to achieve sufficient power. The incidence of thrombosis (1.5%) is lower than values reported in previous studies.[1,3,4] We speculate the lower in the incidence of thrombosis identified in our study may be due to improved surgical techniques and perioperative management. Alternatively, insufficient sample size and/or inaccuracies related to the assessment processes for the occurrence of thrombosis may be implicated as sources of error. Similarly, we lacked sufficient numbers to examine for any relationship between specific cardiac lesions and postoperative thrombotic events. Such a possible association is suggested by the high incidence of thrombotic events in patients following Fontan palliation.[1] Another limitation was our lack of evaluation of quantitative changes in plasma levels of protein C which, as discussed, are decreased post-CPB and may predispose to thrombotic events.[7,14,15,18]

In conclusion, the prevalence of FV_{Leiden} in patients with CHD was not different from that of the population at large. We were unable to make any conclusions with respect to the presence or absence of any association between patients demonstrating FV_{Leiden} and post-CPB thrombotic episodes.

Acknowledgments

Dr. Ellis J. Neufeld is a Fellow of the Louise P. Markey Trust.

References

1 *Dobell ARC, Trusler GA, Smallhorn JF, Williams WG.* Atrial thrombi after the Fontan operation. Ann Thorac Surg 1986; 42: 664–7.

2 *Okita Y, Miki S, Kusuhara K, et al.* Massive systemic venous thrombosis after Fontan operation: report of a case. Thorac Cardiovasc Surg 1988; 36: 234–6.

3 *Franklin WH, Norwood WI.* Management of complications related to the Fontan procedure. *In:* Waldhausen JA, Orringer MB (Eds.). Complications in Cardiothoracic Surgery. St. Louis: Mosby Year Book, 1991: 202–11.

4 *Jahangiri M, Shore D, Kakkar V, Lincoln C, Shinebourne E.* Coagulation factor abnormalities after the Fontan procedure and its modification. J Thorac Cardiovasc Surg 1997; 113: 989–93.

5 *Samaha M, Trossaert M, Conard J, Horellou MH, Elalamy I, Samama MM.* Prevalence and patient profile in activated protein C resistance. Am J Clin Pathol 1995; 104: 450–4.

6 *Koster T, Rosendaal FR, de Ronde H, Briët E, Vandenbroucke JP, Bertina RM.* Venous thrombosis due to poor anticoagulant response to activated protein C: Leiden thrombophilia study. Lancet 1993; 342: 1503–6.

7 *Nuss R, Hays T, Manco-Johnson M.* Childhood thrombosis. Pediatrics 1995; 96: 291–4.

8 *Dahlbäck B, Carlsson M, Svensson PJ.* 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 (USA) 1993; 90: 1004–8.

9 *Bertina RM, Koeleman BPC, Koster T, et al.* Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 1994; 369: 64–7.

10 *Svensson PJ, Dahlbäck B.* Resistance to activated protein C as a basis for venous thrombosis. N Engl J Med 1994; 330: 517–22.

11 *Glauser TA, Rorke LB, Weinberg PM, Clancy RR.* Congenital brain anomalies associated with the hypoplastic left heart syndrome. Pediatrics 1990; 85: 984–90.

12 *Yandava CN, Zappulla DC, Korf BR, Neufeld EJ.* ARMS test for diagnosis of factor V Leiden mutation, a common of inherited thrombotic tendency. J Clin Lab Anal 1996; 10: 414–7.

13 *Tait RC, Walker ID, Reitsma PH, et al.* Prevalence of protein C deficiency in the healthy population. Thromb Haemost 1995; 73: 87–93.
14 *Manno CS, Hedberg KW, Kim HC, et al.* Comparison of the hemostatic effects of fresh whole blood, stored whole blood, and components after open heart surgery in children. Blood 1991; 77: 930–6.
15 *Turner-Gomes SO, Mitchell L, Williams WG, Andrew M.* Thrombin regulation in congenital heart disease after cardiopulmonary bypass operations. J Thorac Cardiovasc Surg 1994; 107: 562–8.
16 *Knöbl PN, Zilla P, Fasol R, Müller MM, Vukovich TC.* The protein C system in patients undergoing cardiopulmonary bypass. J Thorac Cardiovasc Surg 1987; 94: 600–5.
17 *Ankola P, Nardi M, Karpatkin M.* Functional activity of protein C in newborn infants. A report of a study and a review of the literature. Am J Pediatr Hematol Oncol 1992; 14: 140–3.
18 *Boldt J, Knothe C, Schindler E, Welters A, Dapper FF, Hempelmann G.* Thrombomodulin in pediatric cardiac surgery. Ann Thorac Surg 1994; 57: 1584–9.