Clin Orthop Relat Res (2011) 469:131–137
DOI 10.1007/s11999-010-1514-2

SYMPOSIUM: PAPERS PRESENTED AT THE ANNUAL MEETINGS OF THE KNEE SOCIETY

Hematologic Genetic Testing in High-risk Patients Before Knee Arthroplasty

A Pilot Study

Hany Bedair MD, Martin Berli MD, Sefer Gezer MD, Joshua J. Jacobs MD, Craig J. Della Valle MD

Published online: 8 September 2010

Abstract

Background Patients with a personal or familial history of thromboembolism are considered at higher risk for thromboembolic disease after knee arthroplasty. While it remains unclear why some patients develop deep vein thrombosis (DVT) or pulmonary embolism (PE) despite similar operative procedures and the same prophylactic regimen, we presume one explanation would be genetic predisposition.

Questions/purposes We determined the frequency of 12 factors including antithrombin III activity, prothrombin gene mutations, and the presence of phospholipid antibodies in a high-risk patient cohort and compared those findings with the known prevalence in the population at large.

Patients and Methods Patients identified preoperatively as having a personal or familial history of DVT and/or PE were referred for hemostatic serum and genetic tests, including % antithrombin III activity (ATIII), protein C and protein S activities, APC resistance, Factor V gene (Leiden) mutations, prothrombin gene mutations, lupus anticoagulant antibody presence, cardiolipin antibody presence, phosphatidyl antibody presence, β2-glycoprotein antibody presence, and serum homocysteine and lipoprotein(a) levels The frequencies of varying abnormalities were identified and compared to the prevalence reported in the literature.

Results Forty-three of 1944 patients undergoing knee arthroplasty had a history of DVT or PE. Sixteen of 43 (37%) patients had an abnormality and eight of these (19%) had two or more abnormalities. The frequency of nine of the 12 tests appeared to be greater in this cohort than in the population at large.

Conclusions Patients with a personal or familial history of DVT or PE appear to have a high frequency of hereditary prothrombotic abnormalities. Preoperative evaluation by a hematologist may be warranted in patients with a personal or familial history of DVT or PE as the postoperative anticoagulation protocols may be altered and identification

Dr. Jacobs is a consultant for Medtronic Sofamor Danek (Minneapolis, MN) and Zimmer Inc (Warsaw, IN) and receives research/institutional support from Advanced Spine Technologies Inc (San Francisco, CA), Medtronic Sofamor Danek, SpinalMotion Inc (Mountain View, CA), Wright Medical Technologies Inc (Arlington, TN), and Zimmer Inc. Dr. Della Valle is a consultant for Angiotech (Vancouver, BC, Canada), Kinamed (Camarillo, CA), Smith and Nephew Inc (Memphis, TN), and Biomet Inc (Warsaw, IN) and receives research/institutional support from Zimmer Inc.

Each author certifies that his or her institution approved the human protocol for this investigation, that all investigations were conducted in conformity with ethical principles of research, and that informed consent for participation in the study was obtained.

This work was performed at Rush University Medical Center.

H. Bedair (✉)
Department of Orthopaedic Surgery, Harvard Medical School, Boston, MA, USA
e-mail: hbedair@gmail.com

H. Bedair
Massachusetts General Hospital, Boston, MA, USA

H. Bedair
Center for Joint Reconstruction, 2014 Washington Street, Newton, MA 02462, USA

M. Berli, J. J. Jacobs, C. J. Della Valle
Department of Orthopaedic Surgery, Rush University Medical Center, Chicago, IL, USA

S. Gezer
Department of Hematology, Rush University Medical Center, Chicago, IL, USA

of these abnormalities may affect a patient's risk for other disease states.

Level of Evidence Level IV, diagnostic study. See Guidelines for Authors for a complete description of levels of evidence.

Introduction

Knee arthroplasty reliably relieves pain and improves function in patients with end-stage arthropathy of the knee. Among the most common complications after knee arthroplasty is deep vein thrombosis (DVT), and pulmonary embolism (PE) is among the most common causes of death postoperatively [2, 11]. Without either mechanical or pharmacologic prophylaxis, 40% to 60% of patients undergoing knee arthroplasty will develop an asymptomatic DVT detected by imaging studies, 15% to 25% a proximal DVT, and 0.5% to 2% a fatal PE [1, 9]. Multiple risk factors for developing a postoperative DVT have been identified and include advanced age, prolonged immobilization, obesity, and prior history (both personal and familial) of DVT or PE [19]. Moreover, a number of studies have shown hereditary prothrombotic genes and/or hematologic abnormalities lead to hypercoagulable states [3, 8, 12, 18, 19, 22]. The majority of these previous studies have retrospectively observed an increased frequency of one or two abnormalities, such as activated protein C deficiency or hyperhomocysteinemia, in nonorthopaedic patients who have developed a DVT or PE. A single study preoperatively screened all hip and knee arthroplasty patients, regardless of known predisposition, and correlated two abnormalities (prothrombin gene mutation and Factor V Leiden mutation) with an increased incidence of DVT or PE [19]. None of the studies have specifically screened high risk knee arthroplasty patients prior to surgery to determine the presence of genetic mutations and hemostatic or serum abnormalities. Without this knowledge, a controversy will always exist as to the benefit and utility of preoperative screening of patients prior to surgeries (such as knee arthroplasty) that represent a high risk of DVT or PE.

Moreover, it remains unclear why only a minority of patients develop symptomatic DVT or PE events despite similar operative procedures and the same prophylactic regimen. It is unknown if this could be explained by an underlying genetic predisposition. In prior studies investigating genetic predisposition in arthroplasty patients who had a recognized PE postoperatively, four studies identified specific genetic and coagulation abnormalities as independent risk factors and also suggested these tests could be useful in identifying these higher-risk patients preoperatively [10, 14, 16, 20].

Based on these prior studies, the senior author began sending patients with a self-reported personal or familial history of thromboembolic events for evaluation by a hematologist prior to elective knee arthroplasty. Thus, we wanted to determine (1) how frequently an abnormality was identified (2) what changes in the postoperative anticoagulation protocol were recommended and (3) how the observed frequency in this cohort compared with those reported in the population at large.

Patients and Methods

From a group of 1944 patients identified as having a planned primary or revision knee arthroplasty operated on by a single surgeon (CDV) between February 2003 and April 2009, we identified through a routine preoperative questionnaire those patients with a personal or familial history of DVT or PE. Of these 1944 patients, 43 (2.2%) reported having a personal or familial history of DVT or PE. The mean age of the 43 patients was 65.6 years (range, 42–85 years). There were nine men and 34 women (chi square, $p = 0.073$). All 43 patients were referred to a single hematology/coagulation and thrombosis specialist (SG) for evaluation. Thirty-three patients underwent primary TKA, four revision TKA, and four unicompartmental knee arthroplasty.

In addition to a standard medical evaluation, each of the 43 patients had a battery of 12 hemostatic serum and genetic tests based upon the standard workup for hypercoagulability at our institution. These tests included % antithrombin III activity (ATIII), protein C and protein S activities, APC resistance, Factor V gene (Leiden) mutations, prothrombin gene mutations, lupus anticoagulant antibody presence, cardiolipin antibody presence, phosphatidyl antibody presence, β2-glycoprotein antibody presence, and serum homocysteine and lipoprotein(a) levels (Appendix).

A literature search and review was performed to determine the published rates of prevalence of these 12 factors. We included all publications in the English language we found detailing prevalence in any population. Those with large cohorts and relatively well established prevalence rates are reported and any known ethnic variations included. If there was a large variation in rates, the range is reported. Those factors with unknown prevalence rates or sufficiently small populations are reported as "not available" (N/A).

Results

Abnormalities were identified in 16 (37%) of the 43 patients (Table 1). Of the 16 patients having abnormalities, 12 had reported a personal history of DVT or PE and four reported a familial history. There were no patients who reported both. In those patients with abnormalities and a personal history of an event, eight reported a history of

Table 1. Incidence of abnormal test results in tested cohort (n = 16) and in the general population (racial/ethnic prevalence reported when data available)

Defect	Study cohort	Population	Reference
Gene			
Factor V Leiden	18.8% (3)	5.27% • ~5% of Caucasians in N. America • ~2% in Hispanics • ~1% in African-Americans • Rare in Asians	[13]
Prothrombin gene (factor II mutation)	18.8% (3)	2.3%	[12]
Hemostatic			
Anti-thrombin III activity	6.3% (1)	0.02%	[15]
Protein C deficiency	6.3% (1)	0.2–0.4%	[15]
Protein S deficiency	12.6% (2)	0.03–0.13%	[4]
Activated protein C			
Resistance	12.5% (2)	3–7% • ~15% in Scandinavians • Rare in African Americans • Rare in Asians	[21]
Lipoprotein (a)	18.8% (3)	N/A	[7]
Homocysteinemia (elevated serum homocysteine levels)	6.3% (1)	5–10%	[15]
Antibody			
Lupus anticoagulant	25% (4)	3.6%	[17]
Cardiolipin antibody	25% (4)	5%	[17]
Phosphatidylserine antibody	31.3% (5)	4%	[5]
Beta-2 glycoprotein	0%	10%	[6]

DVT, five reported a history of PE, and two patients had both a DVT and PE. The most common abnormality detected was the presence of phosphatidyl antibody in five of the 16 patients with any abnormality (31%). Eight of the 16 patients had a single abnormality detected, 25% had two abnormalities, and 25% had three or more abnormalities identified. The most common finding was a patient reporting a personal history of DVT or PE and having a single mutation (Table 2).

The planned elective surgical procedure was cancelled in two patients based on the preoperative evaluation. One patient was newly diagnosed with leukemia and surgery was cancelled; this patient was heterozygous for Factor V Leiden and also had decreased activated protein C (APC) resistance. The second patient had surgery postponed secondary to the diagnosis of a new, acute DVT preoperatively; her hemostatic tests were normal. Forty-one patients went on to have surgery. Based upon the recommendations of the consulting hematologists who evaluated all tests, 26 (63%) of these patients had a postoperative prophylactic regime that differed from the standard treatment at our institution.

Of the 12 tests performed, abnormalities in nine tests were observed at a higher frequency than the published population prevalence with genetic mutations and the presence of anti-phospholipid antibodies demonstrating the largest absolute differences compared to population prevalence (Table 1).

Table 2. Number of detected abnormalities in those patients reporting a personal history or familial history of DVT or PE

Number of abnormalities	Personal history of DVT/PE (n = 12)	Familial history of DVT/PE (n = 4)
1	6	2
2	3	1
≥3	3	1

Discussion

Despite the routine use of mechanical and/or chemical prophylaxis, which is generally used indiscriminately with similar treatments applied to all patients with little knowledge of each patient's physiologic predisposition [10], the rates of DVT and PE remain substantial [9]. The purpose of this pilot study was to determine the frequency

of specific genetic and hematologic abnormalities in a high-risk patient population scheduled for knee arthroplasty surgery. While it would be ideal to have an inexpensive screening test that would identify patients preoperatively as "higher" or "lower" risk so postoperative anticoagulation could be tailored on an individual basis, such tests are currently not available; however, these data do help identify possible targets for such screening.

We recognize several limitations of this study. First, we lacked testing of patients undergoing similar surgery, but who have no previous history of DVT or PE; therefore, our comparisons of the frequencies of the reported hemostatic abnormalities are indirect and based on previously reported frequencies from other studies. The logistical and cost burden of screening all knee arthroplasty patients would have been too great. Many relatively rare events are usually best investigated with cohort studies, and as this represents a pilot study, we believe the above findings represent an accurate profile of these patients and will hopefully constitute a starting point for further, more detailed analyses. Second, the 12 parameters tested represent the standard hypercoagulability workup at our institution. This does not represent a universally accepted gold standard battery of tests and there may exist multiple other factors not tested for in this study that could have provided relevant information. While a large number of tests could have been undertaken, this does not detract from those reported in this study and it is important to recognize that other abnormalities may exist. Third, the prevalence of these tests may be influenced by ethnic and racial differences among the population and ethnic and racial data were not recorded for this cohort of patients. Because our goal was not to generate specific criteria for preoperative screening, but rather demonstrate the frequency of abnormalities in the group at large, we do not believe this represents a substantial limitation of this study. Finally, it is unclear whether the testing performed in this study was cost-effective or increased quality of care as this study was not randomized and we did not compare DVT or PE rates among a group of patients who did not have such screening. Due to the pilot nature of this study, it was not designed to determine efficacy or cost-effectiveness, and we do not believe this alters the conclusions.

We found that 37% of patients tested had an identifiable abnormality. Moreover, 50% of patients with any abnormality had two or more abnormalities, a finding that previously suggested an even higher increased risk [10, 14]. Several independent studies testing patients who developed PE after an arthroplasty procedure were able to correlate specific hereditary prothrombotic gene abnormalities in these patients when compared to matched controls. In a study identifying 29 patients with PE after THA or TKA, 47% of the matched controls had an identified abnormality, but 100% of patients with PE had an abnormality [10]. Moreover, 47% of patients in the PE group had two or more abnormalities, while only 7% of controls had more than one abnormality. In two similar studies, patients with PE after THA were retrospectively compared with matched controls with respect to genetic polymorphisms [16, 20]. These investigators found an abnormal prothrombin gene, ATIII activity, and/or protein C deficiency correlated to PE after THA. In an additional study, investigators [14] also compared patients who developed PE after THA with matched controls and found a borderline increased odds ratio with a single genetic abnormality, but when combined abnormalities existed, the odds ratio of having a PE after THA was nearly three times that of those with the normal variant. Based on the high frequency of these abnormalities in our and other similar studies, preoperative evaluation by a hematologist may be warranted, although the costs of such testing needs to be considered. While the findings of such an evaluation may affect the orthopaedic procedure performed, it may also have ramifications to the general health of the patient and their children as other health disorders including risk for events such as strokes (REF) and infertility (REF) have been related to these abnormalities. While this may not seem to be the "responsibility" of the orthopaedic surgeon, an awareness of these may be beneficial even if the orthopaedic surgeon recommends that these tests be done by the primary care physician.

Based on the analysis of these tests, the recommended postoperative treatment protocol for DVT/PE prophylaxis was changed in 26 of 41 patients who underwent surgery. The recommendations were made by a consulting hematologist who specializes in thrombotic diseases and while there was no specific protocol for these recommendations, they were based on a wealth of experience in evaluating and synthesizing similar test results in a variety of patient populations. While we do not claim that any one protocol may be superior or even affect the incidence of DVT/PE, these differing recommendations by an experienced clinician in this field highlight another reason to consider preoperative evaluation of patients with a personal or familial history of thromboembolic events (although once again the cost of such testing needs to be considered) as the regimen for postoperative prophylaxis may be altered (including a possible change in the type or duration of prophylaxis) and in some cases a delay in the performance of an elective surgical procedure.

The positive finding of nine of the 12 tests obtained in this study appeared to be greater in this cohort of patients compared to the population at large. The three tests with the greatest absolute difference in prevalence between this cohort and reported population frequencies are lupus anticoagulant, cardiolipin antibody, and phosphatidylserine antibody; all three are implicated in antiphospholipid antibody syndrome. Homocysteinemia and β2-glycoprotein

did not appear to be present at a higher rate in this cohort and may not be as useful a test; however, this observation may be due to a sampling bias related to the relatively low number of patients tested.

Our data demonstrate patients with a personal or familial history of DVT/PE appear to have a high prevalence of hereditary prothrombotic gene abnormalities. Future studies should be randomized, prospective studies directed at determining individual risk for thromboembolic events in all patients with specific hemostatic abnormalities and treatment protocols to address those risks. The ultimate goal would be the ability to stratify all patients, both high and low risk, based upon their genetic profile to initiate optimal prophylaxis for that specific patient.

Appendix

Defect	Gene	Function	Test
Factor V Leiden	F5 gene mutations cause factor V Leiden thrombophilia	Factor V Leiden is the name of a specific mutation that results in thrombophilia, or an increased tendency to form abnormal blood clots in blood vessels	PCR followed by direct sequencing
Prothrombin gene	Prothrombin is encoded by a 21-kb-long gene localized on chromosome 11, position 11 p11-q12	Prothrombin is the precursor of the serine protease thrombin, a key enzyme in the processes of hemostasis and thrombosis, that exhibits procoagulant, anticoagulant, and antifibrinolytic activities	PCR followed by direct sequencing
Anti-thrombin III Activity	Hereditary antithrombin deficiency is caused by mutations in the SERPINC1 gene	2 groups of inhibitory anti thrombin antibodies; those that inhibit coagulation activity and those that inhibit coagulation and amidase activity	Large number of mutations associated with anti-thrombin III activity
Protein C deficiency	Protein C deficiency is caused by mutations in the PROC gene	The main function of protein C is its anticoagulant property as an inhibitor of coagulation factors V and VIII	There are two main types of protein C mutations that lead to protein C deficiency: type I: Quantitative defects of protein C (low production or short protein half life), type II: Qualitative defects, in which interaction with other molecules is abnormal. Defects in interaction with thrombomodulin, phospholipids, factors V/VIII and others have been described
Protein S deficiency	Protein S deficiency is caused by mutations in the PROS1 gene	One of the Vit K dependent anticoagulants. The free form is a cofactor to Protein C in the inactivation of Factors Va and VIIIa	Types I (low plasma levels of total and free protein S), II (functional defect), III (low free protein S)
Homocysteinemia	Variant of the MTHFR gene that leads to a thermolabile variant of this enzyme	Homocysteine is produced when methionine is broken down in the body. Elevated homocysteine levels may cause atherosclerosis and thrombi	Homocysteinemia may be the result of several underlying abnormalities, genetic as well as environmental (low vitamin intake B6, B12, folic acid)
Lupus anticoagulant	LA is a non-specific coagulation inhibitor and a marker for thrombosis	LA is an immunoglobulin that binds to phospholipids and proteins associated with the cell membrane. Since interactions between the cell membrane and clotting factors are necessary for proper functioning of the coagulation cascade, the lupus anticoagulant can interfere with blood clotting as well as in-vitro tests of clotting function. Lupus anticoagulants are also risk factors for thrombosis.	Kaolin clotting time compared to normal plasma as a parameter to define LA activity

Appendix continued

Defect	Gene	Function	Test
Cardiolipin antibody	ACA is a non-specific coagulation inhibitor and a marker for thrombosis	Close relationship to LA	ELISA for polyvalent ACA
Phosphatidylserine antibody	Phosphatidylserine is a phospholipid component, usually found on the cytosolic side of cell membranes. In Apoptosis phosphatidylserine is no longer restricted to the cytosolic part of the membrane, but becomes exposed on the surface of the cell	Tightly associated factor V_a and factor X_a serve as the essential prothrombin-activating complex that assembles on phosphatidylserine (PS)–containing platelet membranes during blood coagulation	ELISA test used for the detection of antibodies to total histone and its subfraction components, and phospholipid antigens, were compared to normal antibody levels
Beta-2 glycoprotein	The gene consists of eight exons, spans 18 kb and is localized on chromosome 17q23–24	Identified as Apolipoprotein H it is required for the recognition of ACA in autoimmune diseases. Only a subset of autoimmune anti-cardiolipin antibodies bind Apo-H, and are associated with increased thrombosis	Important role in the antiphospholipid syndrome (APS) as a cofactor and an (co)antigen in ELISA assays antiphospholipid (aPL) antibodies may require crucial co-factors, amongst which b2-glycoprotein I (b2-GPI)
Activated Protein C resistance	Most common inherited hypercoagulable state associated with venous thrombosis. Caused by a single point mutation in the factor V gene, which predicts the substitution of Arg506 with a Gln. Arg506 is one of three APC-cleavage sites and the mutation results in the loss of this APC-cleavage site	Activated protein C (APC) inhibits coagulation by cleaving and inactivating membrane-bound FVa and FVIIIa. These reactions are potentiated by the non-enzymatic cofactor protein S	Ratio of aPTT with APC:aPTT without APC. Ratio less than two indicates that aPTT is not being significantly prolonged with addition of APC and therefore suggests APC resistance
Lipoprotein (a)	Atherogenic particle that structurally resembles a low density lipoprotein (LDL) particle but contains a molecule of apolipoprotein(a) attached to apolipoprotein B-100 by a disulfide bond	Apo(a) has a high degree of homology with plasminogen, a plasma protein involved in the fibrinolytic process. Because of this homology, Lp(a) may compete with plasminogen and, interfere with the thrombolytic process	Lp(a) is measured in plasma using a commercially available ELISA

References

1. Anderson FA, Jr, Spencer FA. Risk factors for venous thromboembolism. *Circulation.* 2003;107(23 Suppl 1):I9–16.
2. Beksac B, Gonzalez Della Valle A, Salvati EA. Thromboembolic disease after total hip arthroplasty: who is at risk? *Clin Orthop Relat Res.* 2006;453:211–224.
3. den Heijer M, Koster T, Blom HJ, Bos GM, Briet E, Reitsma PH, Vandenbroucke JP, Rosendaal FR. Hyperhomocysteinemia as a risk factor for deep-vein thrombosis. *N Engl J Med.* 1996;334(12): 759–762.
4. Dykes AC, Walker ID, McMahon AD, Islam SI, Tait RC. A study of Protein S antigen levels in 3788 healthy volunteers: influence of age, sex and hormone use, and estimate for prevalence of deficiency state. *Br J Haematol.* 2001;113:636–641.
5. el-Roeiy A, Gleicher N. Definition of normal autoantibody levels in an apparently healthy population. *Obstet Gynecol.* 1988;72: 596–602.
6. Inanc M, Radway-Bright EL, Isenberg DA. Beta 2-glycoprotein I and anti-beta 2-glycoprotein I antibodies: where are we now? *Br J Rheumatol.* 1997;36:1247–1257.
7. Jenner JL, Ordovas JM, Lamon-Fava S, Schaefer MM, Wilson PW, Castelli WP, Schaefer EJ. Effects of age, sex, and menopausal status on plasma lipoprotein(a) levels. The Framingham Offspring Study. *Circulation.* 1993;87:1135–1141.
8. Lane DA, Grant PJ. Role of hemostatic gene polymorphisms in venous and arterial thrombotic disease. *Blood.* 2000;95:1517–1532.
9. Lieberman JR, Hsu WK. Prevention of venous thromboembolic disease after total hip and knee arthroplasty. *J Bone Joint Surg Am.* 2005;87:2097–2112.
10. Mont MA, Jones LC, Rajadhyaksha AD, Shuler MS, Hungerford DS, Sieve-Smith L, Wang P, Cordista AG, Glueck CJ. Risk factors for pulmonary emboli after total hip or knee arthroplasty. *Clin Orthop Relat Res.* 2004;422:154–163.
11. Phillips CB, Barrett JA, Losina E, Mahomed NN, Lingard EA, Guadagnoli E, Baron JA, Harris WH, Poss R, Katz JN. Incidence rates of dislocation, pulmonary embolism, and deep infection during the first six months after elective total hip replacement. *J Bone Joint Surg Am.* 2003;85:20–26.
12. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. 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.* 1996;88:3698–3703.
13. Ridker PM, Miletich JP, Hennekens CH, Buring JE. Ethnic distribution of factor V Leiden in 4047 men and women. Implications for venous thromboembolism screening. *JAMA.* 1997;277:1305–1307.

14. Ringwald J, Berger A, Adler W, Kraus C, Pitto RP. Genetic polymorphisms in venous thrombosis and pulmonary embolism after total hip arthroplasty: a pilot study. *Clin Orthop Relat Res.* 2009;467:1507–1515.
15. Rosendaal FR. Risk factors for venous thrombosis: prevalence, risk, and interaction. *Semin Hematol.* 1997;34:171–187.
16. Salvati EA, Della Valle AG, Westrich GH, Rana AJ, Specht L, Weksler BB, Wang P, Glueck CJ. The John Charnley Award: heritable thrombophilia and development of thromboembolic disease after total hip arthroplasty. *Clin Orthop Relat Res.* 2005;441:40–55.
17. Shi W, Krilis SA, Chong BH, Gordon S, Chesterman CN. Prevalence of lupus anticoagulant and anticardiolipin antibodies in a healthy population. *Aust N Z J Med.* 1990;20:231–236.
18. Svensson PJ, Dahlback B. Resistance to activated protein C as a basis for venous thrombosis. *N Engl J Med.* 1994;330:517–522.
19. Wahlander K, Larson G, Lindahl TL, Andersson C, Frison L, Gustafsson D, Bylock A, Eriksson BI. Factor V Leiden (G1691A) and prothrombin gene G20210A mutations as potential risk factors for venous thromboembolism after total hip or total knee replacement surgery. *Thromb Haemost.* 2002;87:580–585.
20. Westrich GH, Weksler BB, Glueck CJ, Blumenthal BF, Salvati EA. Correlation of thrombophilia and hypofibrinolysis with pulmonary embolism following total hip arthroplasty: an analysis of genetic factors. *J Bone Joint Surg Am.* 2002;84:2161–2167.
21. Zoller B, Garcia de Frutos P, Hillarp A, Dahlback B. Thrombophilia as a multigenic disease. *Haematologica.* 1999;84:59–70.
22. Zoller B, Holm J, Svensson P, Dahlback B. Elevated levels of prothrombin activation fragment 1 + 2 in plasma from patients with heterozygous Arg506 to Gln mutation in the factor V gene (APC-resistance) and/or inherited protein S deficiency. *Thromb Haemost.* 1996;75:270–274.