

Factor V Leiden Does Not Affect Bleeding in Aprotinin Recipients after Cardiopulmonary Bypass

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Background: Carriers of the factor V Leiden mutation (FVL) are resistant to activated protein C proteolysis. Therefore, they are at increased risk of thromboembolic events. Aprotinin is an unspecific proteinase inhibitor frequently used during cardiac surgery procedures to reduce bleeding. However, aprotinin may cause thromboembolic complications after cardiopulmonary bypass (CPB). The primary endpoint of this study was the amount of blood loss after CPB in aprotinin recipients, and secondary endpoints were thromboembolic complications.

Methods: A total of 1,447 consecutive patients who underwent cardiac surgery with CPB were prospectively enrolled. All patients were screened for FVL by a fluorescence-based polymerase chain reaction method. Linear and logistic regression analyses were performed to assess associations of FVL on bleeding and thromboembolic complications.

Results: One hundred seven individuals (7.4%) were heterozygous FVL carriers. No difference was found between FVL carriers and noncarriers regarding age, sex, CPB, type of operation, EuroSCORE, antiplatelet treatment, and reoperation. FVL was not significantly associated with postoperative blood loss, whereas a significant influence was found for female sex ($P < 0.0001$), duration of CPB ($P < 0.0001$), reoperation ($P = 0.001$), and preoperative antiplatelet treatment ($P < 0.002$). Multiple linear regression analysis for total blood loss had an observed power of at least 99%. FVL carriers faced the same risk for postoperative transfusion ($P = 0.391$), reoperation ($P = 0.675$), myocardial infarction ($P = 0.44$), stroke ($P = 0.701$), and 30-day mortality ($P = 0.4$) as did noncarriers.

Conclusions: These data suggest that FVL carriers do not have reduced blood loss compared with noncarriers. Furthermore, the combination of aprotinin and FVL does not enhance the risk for thromboembolic complications.

FACTOR V Leiden (FVL) is an autosomal, single nucleotide polymorphism in the factor V gene, with a prevalence in the European population of 3–7%,¹ whereas it is rarely found in black and Asian populations. FVL has been extensively characterized as the most common known genetic risk factor for deep venous thrombosis.² However, its impact on postoperative bleeding³ and perioperative complications in cardiac surgery are just beginning to be explored.^{4,5}

Factor V Leiden consists of a glutamine substitution for arginine 506, producing a variant that is resistant to inactivation by activated protein C (aPC).⁶ The protein C pathway suppresses excessive activation of blood coagulation and protects against inadvertent blood clots by inactivation of coagulation factors Va and VIIIa.⁷ Therefore, FVL disrupts the activity of this natural anticoagulant pathway leading to prothrombotic risk imbalance.

Cardiac surgery patients have an increased risk for hemorrhagic complications,⁸ as well as for thromboembolic complications such as myocardial and cerebral ischemia.^{9,10} A causal relation between abnormalities in the protein C pathway, such as low plasma aPC levels or aPC resistance, and postoperative complications, such as impaired myocardial performance¹¹ and vein graft occlusion,¹² has previously been suggested. A recent study showed that FVL has a protective effect regarding blood loss and the risk for transfusion in cardiac surgery,³ which is consistent with the observation of a relatively hypercoagulable state after cardiopulmonary bypass (CPB) in FVL carriers.¹³

Aprotinin is widely used in cardiac surgery to diminish postoperative bleeding.¹⁴ It is an unspecific proteinase inhibitor, and some authors found an inhibition of protein C.¹⁵ Recently, it has been postulated that aprotinin is associated with severe postoperative complications after CPB.^{16,17} Other studies suggested that an interaction between aprotinin and FVL might increase the risk for adverse thromboembolic events.^{4,12,18–20} We studied whether there is a cumulative impact of FVL in aprotinin recipients after CPB regarding blood loss, transfusion requirements, and thromboembolic complications. The primary endpoint of this study was the amount of blood loss after CPB in aprotinin recipients, and secondary endpoints were thromboembolic complications.

Materials and Methods

The study protocol was approved by the ethical committee at the Technische Universität München (Ethikkommission der Fakultät für Medizin der Technischen Universität München, Munich, Germany). Written informed consent was obtained from each patient. Between April 2002 and December 2003, we prospectively enrolled a total number of 1,447 consecutive adult patients who underwent cardiac surgery with CPB at our institution. All patients were white. Emergency cases, patients with endocarditis, and patients with aortic aneurysms were excluded.

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Genotyping

Genomic DNA was extracted from whole blood using E.Z.N.A Blood DNA Kit II (PEQLAB Biotechnologie GmbH, Erlangen, Germany). Genotyping for FVL was performed using LightCycler technology (Roche Diagnostics GmbH, Roche Applied Science, Mannheim, Germany) essentially as described.²¹ Primers and hybridization probes were purchased from TIBMOL Biol (TIB MOLBIOL Syntheselabor GmbH, Berlin, Germany). The primer and probe sequences were as follows: forward, 5'-CTT GAA GGA AAT GCC CCA TTA-3'; reverse, 5'-TGC CCA GTG CTT AAC AAG ACC A-3'; wild-type probe, 5'-GGC GAG GAA TAC AGG TAT-FLU; anchor probe, LC Red640-TGT CCT TGA AGT AAC CTT TCA GAA ATT CTG-3'.

Patient Management

Perioperative management and anesthesia were conducted according to standard institutional practice. Preoperative antiplatelet medication was defined when the patient received aspirin and/or clopidogrel as regular medication on the day before surgery. Anticoagulation for CPB consisted of 375 U/kg unfractionated porcine heparin by control of activated clotting time with a target activated clotting time of 480 s. After CPB, anticoagulation was reversed by the application of 1:1 protamine to the dose of heparin.

Aprotinin was given according to a high-dose protocol: Patients received a bolus of 2×10^6 kallikrein inhibitor units (KIU) aprotinin after sternotomy. An additional bolus of 2×10^6 KIU aprotinin was added to the pump prime, and a continuous infusion of 5×10^5 KIU aprotinin per hour was given until chest closure. The decision for transfusion of blood components in the operating room and intensive care unit (ICU) depended on patient age, medical history, coexisting vascular disease, myocardial performance, and the presumed cause of bleeding. Indication for blood transfusion was a hematocrit less than 18% during CPB and less than 24% postoperatively.

Blood loss was measured 6, 12, and 24 h after arrival at the ICU and at the time of tube removal (total chest tube drainage). The total number of administered packed erythrocytes was counted from the time of surgery until hospital discharge.

EuroSCORE

All patients were classified according to the EuroSCORE.²² The EuroSCORE was initiated as a scoring system for the prediction of early mortality in cardiac surgical patients in Europe on the basis of objective risk factors. Risk factors are differently weighed, and each risk factor is validated with a certain amount of points, which are summarized to give a final individual score: The higher the score is, the higher the risk is for early mortality after cardiac surgery.

Patient-related factors were (1) age older than 60 yr (1 point per 5 yr or part thereof), (2) female sex (1 point), (3) chronic pulmonary disease (1 point), (4) extracardiac arteriopathy (2 points), (5) neurologic dysfunction (2 points), (6) previous cardiac surgery (3 points), (7) serum creatinine greater than $200 \mu\text{M}$ (2 points), (8) active endocarditis (3 points), and (9) critical preoperative state (3 points). Cardiac factors were (10) unstable angina on intravenous nitrates (2 points), (11) reduced left ventricular ejection fraction (30–50%: 1 point; < 30%: 3 points), (12) recent myocardial infarction (2 points), and (13) pulmonary systolic pressure greater than 60 mmHg (2 points). Operation-related factors were (14) emergency (2 points), (15) other than isolated coronary surgery (2 points), (16) thoracic aorta surgery (3 points), and (17) surgery for postinfarct septal rupture (4 points).

Postoperative Complications

Patients were closely monitored during their hospital stay by daily clinical checkups and continuous heart rate recording by electrocardiogram. A new onset of a major neurologic deficit, stupor, or coma was defined as stroke.²³

Creatinine was measured preoperatively and on the first postoperative day. Furthermore, the highest reported creatinine level during hospital stay was recorded for each patient.

Postoperative Myocardial Ischemia and Infarction

All patients who underwent isolated coronary artery bypass grafting (CABG) were screened for postoperative myocardial infarction. Creatine kinase (CK) and CK-MB were measured after arrival at the ICU; 4, 8, 24, and 48 h postoperatively; and before hospital discharge. The plasma CK-MB activity was measured by an immunoinhibition assay according to the recommendations of the International Federation of Clinical Chemistry.²⁴ CK-MB values greater than 120 U/l were defined as a marker for myocardial damage and indicated a possible myocardial infarction.²⁵ A 12-lead electrocardiogram was obtained immediately after surgery at the time of arrival at the ICU, on the first postoperative day, before discharge, and whenever required by the clinical course. The diagnosis of a definite myocardial infarction was made by the onset of new and persistent Q waves (≥ 30 ms and ≥ 0.1 mV) in two or more continuous leads of II, III, aVF, or two or more leads of V2–V6, I, and aVL plus a total CK-MB peak greater than 120 U/l according to Greaves *et al.*²⁵

Statistics

Statistical tests were performed with SPSS software 14.0 (SPSS Inc., Chicago, IL). Comparisons between FVL carriers and noncarriers were performed with the use of a *t* test for two independent samples for quantitative data and a chi-square test or Fisher exact test for discrete data as appropriate. Linear and logistic regression analyses

Table 1. Patient Characteristics

Parameter	FVL Carriers (n = 107)	Noncarriers (n = 1,340)	P Value
Age, yr	65.8 ± 11.7	66.3 ± 10.7	0.61
Female sex	35 (32.1%)	431 (31.0%)	0.83
Operation			
CABG	55 (51.4%)	672 (50.1%)	0.61
Valve	35 (32.7%)	401 (29.9%)	0.55
CABG plus valve	12 (11.2%)	196 (14.6%)	0.33
Others	6 (5.6%)	71 (5.3%)	0.71
CPB duration, min	101.2 ± 34.7	104.3 ± 41.7	0.45
Previous cardiac surgery	6 (5.6%)	79 (5.9%)	0.90
EuroSCORE according to Nashef <i>et al.</i> ²²	4.37 ± 2.6	4.42 ± 2.7	0.86
Creatinine,* mg/dl	1.11 ± 0.25	1.13 ± 0.5	0.18
Antiplatelet treatment			
Within 5 days before surgery	47 (43.9%)	595 (44.4%)	0.92
< 24 h before surgery	19 (17.8%)	159 (11.9%)	0.07
LV function (ejection fraction)	61.5% ± 13.2	59.1% ± 14.9	0.13

* Creatinine measured preoperatively.

CABG = coronary artery bypass grafting; CPB = cardiopulmonary bypass.

were performed to assess association of FVL with clinical parameters. *P* values were two-sided and subject to a significance level of 5%.

Results

One hundred seven patients (7.4%) were found to be heterozygous for FVL, whereas no patient was homozygous, which is consistent with the reported prevalence of FVL in the white population.²⁶ Demographic parameters and common risk factors for postoperative complications were similar between FVL carriers and noncarriers (table 1).

Blood Loss

Multiple linear regression analysis for total blood loss showed statistically significant effects for female sex (*P* < 0.0001), the duration of CPB (*P* < 0.0001), reoperation (*P* = 0.001), and preoperative antiplatelet treatment (*P* < 0.002). EuroSCORE (*P* = 0.106), FVL (*P* = 0.907), and age (*P* = 0.702) did not affect postoperative bleeding (table 2). In FVL carriers, total blood loss was not significantly different (mean 658 ± 521 *vs.* 666 ± 536 ml; *P* = 0.886). With a sample size of *n* = 1,447, the multiple linear regression test for the eight covariates included had an observed power of at least 99% to detect *R*² of 0.09 for a prespecified significance level of 5%. Similarly, blood losses at 6, 12, and 24 h postoperatively were not significantly different between the two groups (fig. 1).

Transfusion Requirements

The percentages of patients receiving blood components (packed erythrocytes, platelets, and/or fresh frozen plasma) were 40.4% in FVL carriers and 35.9% in noncarriers (*P* = 0.355) during hospital stay. Accordingly, no significant differences were observed when the

blood components were analyzed individually (packed erythrocytes: 40.4% *vs.* 34.4%, *P* = 0.211; fresh frozen plasma: 9.2% *vs.* 9.8%, *P* = 0.820; platelets: 5.8% *vs.* 4.8%, *P* = 0.647; fig. 2).

Postoperative Complications

In a subgroup of 727 patients who underwent isolated CABG, there were no differences between FVL carriers and noncarriers in the number of grafts per patient (2.6 ± 0.6 in both groups), the percentage of venous grafts (67.2% of the FVL carriers and 60.2% of the noncarriers; *P* = 0.303), and the percentages of internal mammary artery bypass grafts (98.2% *vs.* 94.8%; *P* = 0.226) and radial artery bypass grafts (34.5% *vs.* 30.5%; *P* = 0.470). All patients were screened for postoperative myocardial infarction as described.²⁵ The incidence of postoperative myocardial infarction was not significantly greater in FVL carriers compared with noncarriers (5.5% *vs.* 3.4%; *P* = 0.440; table 3). No increased occurrence of a hemorrhagic complication such as reoperation (2.8% *vs.* 2.2%, FVL *vs.* noncarriers; *P* = 0.675) or thromboembolic complications such as postoperative stroke (1.9% *vs.* 2.5%; *P* = 0.701) were observed in FVL carriers. The proportion of patients with a prolonged stay on the ICU (postoperative ventilation > 24 h) was similar in both

Table 2. Linear Regression Model for Total Blood Loss

Clinical Parameter	Coefficient B	SEM	P Value
Constant	538.038	109.692	0.001
Previous cardiac surgery	206.033	63.338	0.001
Antiplatelet treatment	86.475	28.358	0.002
EuroSCORE	11.519	7.121	0.106
Duration of CPB	2.976	0.363	0.000
Age	−0.626	1.635	0.702
Factor V Leiden	−6.215	52.968	0.907
Female sex	−183.209	31.610	0.000

CPB = cardiopulmonary bypass.

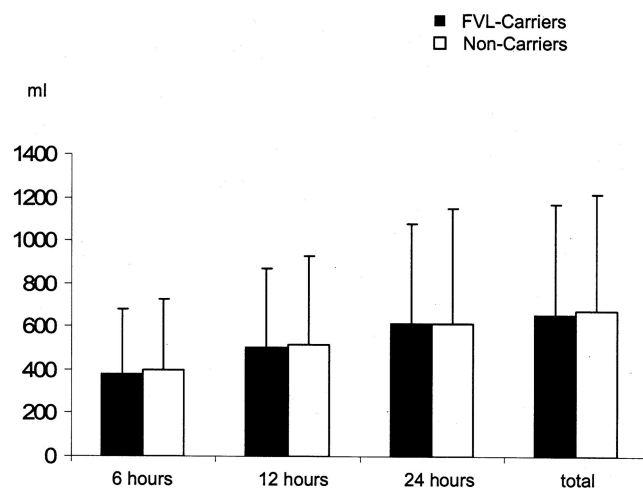


Fig. 1. Effect of factor V Leiden (FVL) on postoperative blood loss after 6, 12, and 24 h and in total. Bars denote mean blood loss by chest tube output; error bars indicate the standard deviations. No significant difference was found between FVL carriers and noncarriers.

groups (8.4% vs. 4.5%; $P = 0.071$), and no significant difference was found in 30-day mortality (4.6% vs. 3.8%; $P = 0.400$; table 3).

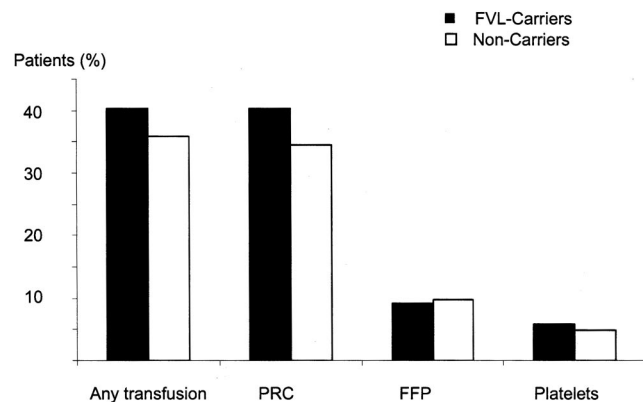


Fig. 2. Effect of factor V Leiden (FVL) on transfusion. Bars denote the percentage of patients who received transfusion during their hospital stay. The percentages of patients receiving blood components were 40.4% in FVL carriers and 35.9% in noncarriers ($P = 0.355$). Accordingly, no significant differences were observed when the blood components were analyzed individually (packed red cells [PRC]: 40.4% vs. 34.4%, $P = 0.211$; fresh frozen plasma [FFP]: 9.2% vs. 9.8%, $P = 0.820$; platelets: 5.8% vs. 4.8%, $P = 0.647$).

Postoperative Creatinine Levels

Multiple linear regression analysis for postoperative creatinine levels showed statistically significant effects for female sex ($P = 0.003$), duration of CPB ($P < 0.001$), age ($P = 0.006$), preoperative creatinine levels ($P < 0.001$), and reoperation ($P = 0.001$). In FVL carriers, postoperative creatinine levels were not significantly different from noncarriers ($P = 0.902$). With a sample size of $n = 1,447$, the multiple linear regression test for the eight covariates included had an observed power of at least 99% to detect R^2 of 0.688 for a prespecified significance level of 5%.

Discussion

There are only a few studies investigating genetic risk factors in cardiovascular surgery, and most of them were performed in a small number of patients only. On the other hand, inherited thrombophilic polymorphisms are common in the white population, and the understanding regarding those genetic variants is only beginning to be explored. A recent study reported a beneficial effect of FVL on perioperative blood loss and transfusion requirements.³ Furthermore, an effect of FVL on perioperative risk was previously suggested.^{18,19,27}

Blood Loss

A recent study in 517 patients undergoing cardiac surgery reported an effect of FVL on perioperative blood loss and transfusion requirements. The authors found that in FVL carriers, blood loss was approximately 30% less than in noncarriers.³ These data are in contrast to our findings. We did not find a significant association of FVL on perioperative blood loss or transfusion requirements. The differences between these studies may be explained by the fact that all of our study population received aprotinin, whereas only 22% of the patients in the study published by Donahue *et al.* were treated with aprotinin and 37% of the patients were treated with aminocaproic acid. Therefore, we conclude that the administration of aprotinin after CPB might have concealed potential blood-sparing effects of FVL in our patients. Moreover, the regression analysis for total blood loss had an observed power of at least 99% to detect R^2 of 0.09 for a prespecified significance level of 5%.

Table 3. Risk Stratification for the Postoperative Course

Parameter	Number of FVL Carriers to Achieve a Power of 80%	FVL Carriers	Noncarriers	Power, %	P Value
Myocardial infarction*	7 (12.5%)	3 (5.5%)	23 (3.4%)	18	0.440
Possible myocardial infarction*	10 (17.5%)	4 (7.7%)	42 (6.5%)	6	0.769
Reoperation for bleeding	8 (7.7%)	3 (2.8%)	29 (2.2%)	6	0.675
Stroke	9 (8.0%)	2 (1.9%)	33 (2.5%)	4	0.701
Postoperative ventilation > 24 h	9 (8.5%)	9 (8.4%)	60 (4.5%)	12	0.071
30-Day mortality	11 (10.3%)	5 (4.6%)	53 (3.8%)	7	0.400

* Only patients with isolated coronary artery bypass grafting.

FVL and Aprotinin

Although antifibrinolytic therapy is well established to decrease the hemorrhagic risk associated with CPB, the possible prothrombotic risks of these drugs have been discussed, especially in FVL carriers. A recent multicenter study suggested an elevated risk for perioperative complication in aprotinin recipients after cardiac surgery.²⁸ In addition to the inhibition of fibrinolysis, the use of aprotinin in FVL carriers might compromise the protein C regulatory pathway resulting in a thromboembolic event. It has been previously shown *in vitro* that aprotinin may induce resistance to aPC in normal plasma and exacerbates aPC resistance in the plasma of FVL heterozygotes.¹⁵ Some reports on the use of antifibrinolytic therapy in FVL carriers with fatal results have been published.^{18–20,29} In a recent case report, the authors conclude that they will withhold antifibrinolytic therapy in any patient carrying the FVL mutation.¹⁸

FVL and Postoperative Complications

In this study, the onset of postoperative stroke and the occurrence rate of postoperative myocardial infarction served as indicators for severe ischemic complications during hospital stay after cardiac surgery. Despite intraoperative myocardial protection and improvements in surgical techniques, local transient ischemia still occurs during CABG. Only a minority of patients experience a postoperative myocardial infarction.²⁵ A possible association of FVL with graft occlusion was assessed previously by performing coronary angiography in 100 patients 3 months after elective surgery.¹² Graft occlusion was observed in 45% of FVL carriers (5 of 11) compared with 20% (18 of 89) of noncarriers. Although these results were of borderline statistical significance ($P = 0.06$), they are well in accordance with a recent case report describing complete coronary artery bypass graft occlusion 1 month after surgery in a FVL carrier.³⁰ To evaluate the risk for postoperative complications in FVL carriers receiving antifibrinolytic therapy, we chose the incidence of postoperative myocardial infarction in patients who underwent isolated CABG. Even if—by definition—postoperative myocardial infarction can not be directly correlated to early bypass occlusion, it remains the most reliable substrate for severe ischemic myocardial damage after surgery.²⁵ We did not find a significant difference in the incidence of postoperative myocardial infarction and 30-day mortality between FVL carriers and the control group. On the other hand, our study protocol did not include cardiac catheterization after CABG; hence, we could not demonstrate graft patency after surgery. We can only rule out deleterious effects of FVL on early graft patency leading to a significantly higher rate of postoperative myocardial infarctions. However, long-term effects of FVL on the graft patency rate, especially in vein grafts, still await investigation.

As implicated in table 3, the current study is under-

powered to evaluate possible associations of FVL with postoperative complications in aprotinin recipients after CPB. However, to our knowledge, the current study screens for FVL the largest number of patients who underwent CPB in the presence of aprotinin, so that we think that our results are noteworthy.

Conclusions

Our data suggest that FVL carriers show neither reduced blood loss nor reduced transfusion requirements compared with noncarriers. Furthermore, the results imply that FVL carriers do not have an increased risk to develop ischemic myocardial complications, even in the presence of aprotinin. Studies in even larger samples are necessary to definitely rule out potential deleterious effects of the combination FVL carrier/aprotinin recipient.

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