Clinical and Applied Thrombosis/Hemostasis
Volume 16 Number 1
January/February 2010 66-70

10.1177/1076029608320721
http://cath.sagepub.com

Type and Location of Venous Thromboembolism in Carriers of Factor V Leiden or Prothrombin G20210A Mutation Versus Patients With No Mutation

Mirjana Kovac, MD, MSc, Gorana Mitic, MD, PhD, Zeljko Mikovic, MD, PhD, Nebojsa Antonijevic, MD, PhD, Valentina Djordjevic, MSc, Danijela Mikovic, MD, MSc, Vesna Mandic, MD, PhD, Ljiljana Rakicevic, MSc, and Dragica Radojkovic, PhD

Factor V Leiden (FVLeiden) and prothrombin G20210A are the most common genetic causes of thrombophilia and established risk factors for different clinical manifestations of venous thromboembolism (VTE). This study investigated whether the clinical manifestation of VTE, the extension of deep vein thrombosis (DVT) and the presence of transient risk factors at the time of the first VTE, differed among patients with mutations (97 with FVLeiden; 33 with prothrombin G20210A) and in 109 patients without thrombophilia. Isolated pulmonary embolism (PE) was less prevalent in patients with FVLeiden (6%) and no thrombophilia (6%) than in those with prothrombin G20210A (15%). No difference was found in the incidence of distal DVT. Regarding the extension of proximal DVT, the lowest incidence for isolated popliteal vein and the highest for iliofemoral vein were observed in patients with prothrombin G20210A. No difference was observed between groups of patients with or without thrombophilia by unprovoked VTE. The pregnancy/puerperium was the most prevalent risk factor in carriers of prothrombin G20210A. Among FVLeiden carriers, the most prevalent risk factor was surgery, and in patients without thrombophilia, it was trauma ($P < .05$). Thrombosis of the upper limb was more frequent in a group without thrombophilia than in patients with mutations ($P < .01$). Transverse sinus venous thrombosis was present only in patients with prothrombin G20210A. Carriers of prothrombin G20210A have an increased risk of developing isolated PE and more severe clinical manifestations than those with FVLeiden or without thrombophilia.

Keywords: factor V Leiden; prothrombin G20210A; venous thromboembolism; clinical manifestations; isolated PE

The most common genetic causes of thrombophilia are two mutations in the genes coding for coagulation factor V and prothrombin, G→A substitution at gene position 1691 (factor V Leiden or FVLeiden), and the noncoding G→A mutation at gene position 20210 (prothrombin G20210A), respectively. The former mutation renders activated FV partially resistant to the inactivation by its naturally occurring anticoagulant protein C, and prothrombin mutation is associated with increased plasma levels of prothrombin to approximately 30%.[1-3]

From the Blood Transfusion Institute of Serbia, Belgrade, Serbia (MK, DM); Institute of Laboratory Medicine, Clinical Center of Vojvodina, Novi Sad, Serbia (GM); Gynaecology and Obstetrics Clinic Narodni Front, Belgrade, Serbia (ZM, VM); Institute of Cardiovascular Diseases, Clinical Centre of Serbia, Belgrade (NA); and Institute of Molecular Genetics and Genetic Engineering, Belgrade, Serbia (VD, LR, DR).

This work was supported by grant 143051 from the Ministry for Science, Serbia.

Address correspondence to: Valentina Djordjevic, Institute of Molecular Genetics and Genetic Engineering, Vojvode Stepe 444A, P.O. Box 23, 11010 Belgrade, Serbia; e-mail: pg20210@eunet.yu.

Both mutations are correlated with an increased amount of thrombin formation and are established risk factors for different clinical manifestation of venous thromboembolism (VTE).[4,5] Prothrombin G20210A is associated with an increased risk of portal vein thrombosis, and more severe clinical manifestation than those in FVLeiden or no thrombophilia.[6] Several studies showed a lower incidence of FVLeiden in patients with symptomatic isolated pulmonary embolism (PE).[7,8] There are two main hypotheses for the FVLeiden paradox which explain risk of DVT but not of PE. The first is that it is due to selection bias, and the other hypothesis is that the thrombus is more stable and more adherent to the vessel wall in patients with FVLeiden, perhaps because this factor enhances local thrombin generation and intensifies the local inflammatory process to the thrombus.[9] Contrary to these findings other investigators reported the similar prevalence of FV Leiden and prothrombin G20210A mutation among patients with isolated PE and control subjects.[10]

In our previous study, we observed no difference considering the rate of recurrence of VTE between 2 groups of patients with mutations. In FV Leiden group the estimated relative risk of recurrence was 1.67 and in group with prothrombin G20210A was 1.65.[11,12] The purpose of this study was to investigate whether the clinical manifestation of VTE considering: isolated DVT, PE with DVT, and isolated PE were different between patients with mutations of FVLeiden or prothrombin G20210A. We also investigated the extension of DVT and the presence of transient risk factors at the time of first VTE in these groups of patients.

Study Design

Patients

From June 1998 to June 2007, 614 patients were referred in our Center for Hemostasis Research to be investigated for thrombophilia screening after developing of symptomatic VTE. Anamnestic data were recorded from all the participants on the presence of the following transient risk factors in the month preceding thrombosis: surgery, pregnancy/puerperium, trauma, infections, oral contraceptive or hormone replacement therapy use, or long sitting position. In the absence of the risk factors, thrombosis was considered unprovoked. Review of the medical test results and records (color duplex ultrasonography, venography, scintigraphy) revealed the size and location of thrombosis. DVT of the veins of the calf were considered distal and those involving the vein segments (popliteal, femoral, external iliac, internal iliac, common iliac, or inferior cava) were considered proximal. We also included the patients with unusual forms of VTE likewise thrombosis of subclavio-brachialis vein, mesenterialis, lienalis, and transverse sinus venous thrombosis.

A total of 309 patients were excluded from the study as they had no medical records, or they had antithrombin, protein C, protein S deficiency, antiphospholipid antibodies, or combined abnormalities. The patients with diabetes or malignancy diseases were also excluded. In total, 245 patients were included in the study, divided into 3 groups: 97 with FVLeiden, 39 with prothrombin G20210A mutation, and 109 patients without thrombophilia. The study protocol was approved by local research ethics committee, and informed consent was obtained from all participating patients.

Laboratory Methods

Plasma samples were taken from each patient for the hemostasis screening tests: prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT), fibrinogen and number of platelets, diagnostic tests for thrombophilia: antithrombin, protein C, protein S, presence of lupus anticoagulans (LA), and activated protein C resistance (APC-R). For the detection of thrombophilia IL tests (Instrumentation Laboratory, Milan, Italy) were used, and analyses were performed on IL Coagulometer ACL 6000. FV Leiden and FII G20210A mutations were detected by polymerase chain reaction as previously described.[13]

Statistical Analysis

The χ^2 test or Fisher's exact test was used to detect differences in the distribution of categorical variables (type of VTE, extension of DVT, risk factors, and male to female ratio). *P* values less than .05 were considered statistically significant. The analyses were performed using the SPSS package version.

Results

Of the 245 patients with VTE 227 (92%) had DVT, which in 24 (10%) was complicated by symptomatic

Table 1. Type of Venous Thromboembolism and Transient Risk Factors of VTE in the Study Population

	Factor V Leiden	FII G20210A	Without Mutations	*P*
n	97	39	109	
M/F	37/60	10/29	30/79	.18
Median age in years at thrombosis (range)	33 (17-63)	34 (16-67)	33 (17-60)	.87
Isolated DVT, n (%)	82 (84)	32 (82)	89 (82)	.85
DVT/PE, n (%)	10 (10)	1 (3)	13 (12)	.02
Isolated PE, n (%)	5 (6)	6 (15)	7 (6)	.11
Unprovoked DVT, n (%)	31 (32)	15 (39)	47 (43)	.26
Provoked DVT, n (%)	66 (68)	24 (62)	62 (57)	.26
Surgery	15 (23)	2 (8)	12 (19)	.03
Pregnancy/puerperium	36 (54)	18 (75)	27 (44)	.03
Trauma	6 (9)	1 (5)	12 (19)	.04
Infections	6 (9)	1 (4)	6 (10)	.58
Long sitting position	2 (3)	—	4 (6)	.40
Hormonal therapy	1 (2)	2 (8)	1 (2)	.50

NOTES: M/F = male/ female; DVT = deep venous thrombosis; PE = pulmonary embolism; VTE = venous thromboembolism.

Table 2. Incidence and Localization of DVT in Patients With and Without Mutations

	Factor V Leiden	FII G20210A	Without Mutations	*P*
n	92	33	102	
Distal (of the calf) DVT, n (%)	6 (7)	2 (6)	9 (9)	.32
Proximal DVT, n (%)	81 (88)	24 (73)	70 (69)	.01
Isolated popliteal vein	36 (39)	3 (9)	40 (39)	.004
Isolated femoral vein	5 (5)	4 (12)	5 (5)	.30
Popliteal/femoral	9 (10)	3 (9)	6 (6)	.71
Popliteal/iliofemoral vein	1 (1)	1 (3)	2 (2)	.34
Iliofemoral vein	27 (29)	13 (40)	15 (15)	.01
Isolated iliac vein	1 (1)	—	2 (2)	
Isolated inferior cava	1 (1)	—	—	
Unusual forms of VTE, n (%)	5 (5)	7 (21)	23 (23)	
Subclavioaxilaris vein	4 (4)	3 (9)	23 (23)	.01
Sinus transverses	—	3 (9)	—	
Mesenterialis	1 (1)	—	—	
Lienalis	—	1 (3)	—	

NOTES: DVT = deep venous thrombosis; VTE = venous thromboembolism.

PE, and 18 patients (8%) had isolated PE. The median age at thrombosis was 33 years (range 16-67). In 93 patients (38%) VTE was unprovoked. In all, 97 patients carried FVLeiden, 39 patients carried prothrombin G20210A, and 109 patients were without thrombophilia. Table 1 shows the general characteristics, type of venous thromboembolism and presence of transient risk factors at the time of thrombosis according to the presence or absence of thrombophilia. No difference was found between the 3 groups according to the median age at the time of the first thrombosis. Regarding the presence of isolated DVT or DVT/PE among 3 groups, no difference was observed. Among patients with isolated PE, we found a higher rate of PE incidence in patients with prothrombin G20210A (15%) than in those with FVLeiden (6%) and without thrombophilia (6%), but without statistical significance. No difference was observed between groups of patients with or without thrombophilia with unprovoked VTE. Considering the presence of transient risk factors in the group with prothrombin G20210A the most prevalent one was pregnancy/puerperium, in the group with FVLeiden surgery, and in patients without thrombophilia trauma. Table 2 shows the extension of DVT in patients with or without thrombophilia. No difference was found regarding the incidence of distal

DVT. Considering the extension of proximal DVT, the lowest incidence for isolated popliteal vein (9%) and the highest for iliofemoral vein (40%) were found in patients, carriers of prothrombin G20210A mutation. The unusual forms of VTE thrombosis of upper limb were significantly more frequently present in the group without thrombophilia (77%) than in those with FVLeiden (13%) or with prothrombin G20210A (10%; $P < .01$). The transverse sinus venous thrombosis was only present in patients with prothrombin G20210A mutation, and in 2 of 3 patients, thrombosis developed during pregnancy/puerperium.

Discussion

Venous thromboembolism has a wide spectrum of clinical manifestations and many factors could play different role in determining the various locations of DVT and its major complication—PE. The presence of thrombophilia could give a possible explanation regarding the genetic predisposition for development of VTE. The most common genetic causes of thrombophilia are 2 mutations in the genes coding for coagulation factor V and prothrombin.[1-3] Both mutations are correlated with an increased amount of thrombin formation and are established risk factors for VTE.[4,5]

In our study, the clinical manifestation (presence of isolated DVT, DVT/PE, or isolated PE), extension of DVT and the presence of transient risk factors were retrospectively investigated in patients with FVLeiden, with prothrombin G20210A mutations and patients without thrombophilia. Our results showed no difference among three groups according to median age at the time of the first thrombosis. Contrary to our findings, Martinelli et al[14] recently reported a younger age (24 years) at the first VTE in patients with prothrombin G20210A mutation. Regarding the presence of isolated DVT or DVT/PE among the 3 groups, no difference was observed. Among the patients with isolated PE, a higher rate of incidence of PE was found in patients with prothrombin G20210A (15%) than in those with FVLeiden (6%) or without thrombophilia (6%), but it was not statistically significant. Emmerich et al[15] and de Moerloose et al[16] also confirmed the lower frequency of FVLeiden in patients with pulmonary embolism in comparison with patients with DVT without PE. The prothrombin G20210A mutation was equally balanced in both patient groups. Contrary to these findings, Margagline et al[10] reported similar prevalences of FV Leiden and prothrombin G20210A mutations among patients with isolated PE and control subjects. In our study, no difference was observed between groups of patients with or without thrombophilia with unprovoked VTE. Regarding the presence of transient risk factors in group with prothrombin G20210A, the most prevalent observed risk factor was pregnancy/puerperium, in the group with FVLeiden surgery, and in the group without thrombophilia trauma. Our results are in concordance with the study of Wahlander et al,[17] where the tendency toward increased risk of VTE was found with the FVLeiden mutation during orthopedic surgery. On the contrary, Martinelli et al[14] found no difference between patients with and without mutations, considering the type of transient risk factors. Among our patients, no difference was found in the incidence of distal DVT. Similar results were observed by Schulman,[18] who reported statistically no significant difference in proportion of any of the defects tested between distal and proximal DVT in patients with and without thrombophilia. Opposite to our findings, Martinelli et al[14] reported that patients without thrombophilia had higher rate of distal DVT than patients with mutations. Regarding the extension of proximal DVT, in our study, the lowest incidence for isolated popliteal vein (9%) and the highest for iliofemoral vein (40%) were found in patients with prothrombin G20210A. In the group with FVLeiden, the most frequent extension of DVT were found in popliteal vein (39%) and lower in iliofemoral vein. The lower incidence of DVT in iliofemoral veins among patients with FVLeiden and highest in group with G20210A could give a possible explanation for lower incidence of PE in FVLeiden and higher in G20210A group. Björgell et al[19] showed the low incidence of iliofemoral DVT in patients with FVLeiden, and study by Huisman et al[20] observed association of FVLeiden mutation with more distal location of vein thrombosis in the leg. Regarding the presence of unusual forms of VTE, our study reports more frequent thrombosis of the upper limb in the group without thrombophilia (77%) than in patients with FVLeiden (13%) or with prothrombin G20210A (10%; $P < .01$). Our results also showed that thrombophilia is less frequent in upper limb than in lower limb DVT. Studies by Prandoni et al[21] and Martinelli et al[22] reported the prevalence of FVLeiden below 10% in patients with upper limb thrombosis. In our study, we observed the presence of the transverse sinus venous thrombosis only in the prothrombin mutation group, and in 2 of 3 patients, thrombosis developed during

pregnancy/puerperium. It has been shown that among transient risk factors, the use of oral contraceptives (in up to 85% to 95% of women) as well as the pregnancy and puerperium (30% to 80%) play crucial role in development of cerebral vein thrombosis.[23,24]

In conclusion, our study showed that carriers of prothrombin G20210A had an increased risk of developing isolated PE and more severe clinical manifestation than those with FVLeiden or without thrombophilia. Further studies are needed to explain the possible implications on the treatment of patients who are the carriers of these mutations.

References

1. Rosendaal FR. Risk factors for venous thrombotic disease. *Thromb Haemost.* 1999;82:610-619.
2. Bertina RM. Genetic approach to thrombophilia. *Thromb Haemost.* 2001;86:92-103.
3. Dahlbäck B. Resistance to activated protein C caused by the Factor VR506Q mutation is a common risk factor for venous thrombosis. *Thromb Haemost.* 1997;78:483-488.
4. Poort SR, Rosendal 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.
5. Martinelli I, Bottasso B, Duca F, Faioni E, Mannucci PM. Heightened thrombin generation in individuals with resistance to activated protein C. *Thromb Haemost.* 1996;75:703-705.
6. Janssen HL, Meinardi JR, Vleggaar FP, et al. Factor V Leiden mutation, prothrombin gene mutation and deficiencies in coagulation inhibitors associated with Budd-Chiari syndrome and portal vein thrombosis: results of a case-control study. *Blood.* 2000;96:2364-2368.
7. Manten B, Westendorp RG, Koster T, Reitsma PH, Rosendaal FR. Risk factor profiles in patients with different clinical manifestations of venous thromboembolis: a focus on the factor V Leiden mutation. *Thromb Haemost.* 1996;76:510-513.
8. Martinelli I, Cattaneo M, Panzeri D, Mannucci PM. Low prevalence of factor V:Q506 in 41 patients with isolated pulmonary embolism. *Thromb Haemost* 1997;77: 440-443.
9. Bounameaux H. Factor V Leiden paradox: risk of deep vein thrombosis but not of pulmonary embolism. *Lancet.* 2000;356:182-183.
10. Margaglione M, Brancaccio V, De Lucia D, et al. Inherited thrombophilic risk factors and venous thromboembolism: distinct role in peripheral deep venous thrombosis and pulmonary embolism. *Chest.* 2000;118: 1405-1411.
11. Kovac M, Mikovic D, Antonijevic N, et al. FV Leiden mutation and risk of recurrent venous thromboembolism in Serbian population. *J Thromb Thrombolysis.* 2008;25: 284-287.
12. Kovac M, Mikovic D, Antonijevic N, et al. Risk of recurrent venous thromboembolism in patients with deep vein thrombosis who are carriers of the Factor V Leiden or prothrombin gene mutation. *Pathophysiol Haemost Thromb.* 2006;35:P-1067.
13. Djordjevic V, Rakicevic LJ, Spasic M, et al. FV Leiden, FII G20210A, MTHFR C677T mutations and first venous thrombosis during pregnancy and puerperioum. *Vojnosanit Pregl.* 2004;62:201.
14. Martinelli I, Battaglioli T, Razzari C, Mannucci PM. Type and location of venous thromboembolism in patients with factor V Leiden or prothrombin G20210A and in those with no thrombopholia. *J Thromb Haemost.* 2007;5:98-101.
15. Emmerich J, Rosendaal FR, Cattaneo M, et al. Combined effect of factor V Leiden and prothrombin 20210 A on the risk of venous thromboembolism-pooled analysis of 8 case-control studies including 2310 cases and 3204 controls. Study Group for Pooled-Analysis in Venous Thromboembolism. *Thromb Haemost.* 2001;86:809-816; erratum 1598.
16. de Moerloose P, Reber G, Perrier A, Perneger T, Bounameaux H. Prevalence of factor V Leiden and prothrombin G20210A mutations in unselected patients with venous thromboembolism. *Br J Haematol.* 2000;110: 125-129.
17. Wåhlander K, Larson G, Lindahl TL, et al. Factor V Leiden (G169A) 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.
18. Schulman S. Thrombophilia and location of venous thromboembolism. *J Thromb Haemost.* 2007;5:2151-2152.
19. Björgell O, Nilsson PE, Nilsson JA, Svensson PJ. Location and extent of deep vein thrombosis in patients with and without FV:R 506Q Mutation. *Thromb Haemost.* 2000;83:648-651.
20. Huisman MV, Klok FA, Karami Djurabi R, Tormene D, Simioni P, Prandoni P. Factor V Leiden is associated with more distal location of deep vein thrombosis in the leg. *J Thromb Haemost.* 2008;6:544-545.
21. Prandoni P, Polistena P, Bernardi E, et al. Upper-extremity deep vein thrombosis. Risk factors, diagnosis, and complications. *Arch Intern Med.* 1997;157:57-62.
22. Martinelli I, Cattaneo M, Panzeri D, Taioli E, Mannucci PM. Risk factors for deep venous thrombosis of the upper extremities. *Ann Intern Med.* 1997;126:707-711.
23. Legnani C, Cosmi B, Valdrè L, et al. Venous thromboembolism, oral contraceptives and high prothrombin levels. *J Thromb Haemost.* 2003;1:112-117.
24. Martinelli I, Sacchi E, Landi G, Taioli E, Duca F, Mannucci PM. High risk of cerebral-vein thrombosis in carriers of prothrombin gene mutation and in users of oral contraceptives. *N Engl J Med.* 1998;338:1793-1797.