Activated Protein C Resistance in Polycythemia Vera

Alp GÜNAY*, Ahmet ÖZTÜRK*, Tülin BUDAK**, Uður ÖZBEK***, Necdet ÜSKENT*

   * Department of Haematology-Oncology, GATA Haydarpaþa Training Hospital,
  ** Department of Haematology, Marmara University Hospital,
 *** Institute For Experimental Medicine Department of Genetics, Ýstanbul, TURKEY

ABSTRACT

Activated protein C resistance is a result of a point mutation in factor V gene (Leiden mutation) and can be identified in approximately 50% of patients with thrombosis, making it an important risk factor for thrombosis. The aim of this study is to evaluate the role activated protein C resistance in the hypercoagulable state seen in polycythemia vera. We compared patients with polycythemia vera (n: 24) for increased risk of thromboembolism and activated protein C resistance, with the results of patients with chronic myelogenous leukemia (n: 27) and healthy control group (n: 52). Activated protein C resistance test and factor VIII activity was determined by an aPTT based test. Anticardiolipin antibodies IgG and IgM were also determined by ELISA. Leiden mutation was studied with polymerase chain reaction. Venous thromboses were observed in 12.5% and arterial thromboses in 41.6% of patients with polycythemia vera. Arterial thromboses were recognized in 7.4% of patients with chronic myelogenous leukemia. Activated protein C resistance was identified in 20.8% of patients with polycythemia vera and 14.8% with chronic myelogenous leukemia (versus 1.8% of healthy control subjects). The risk of thrombosis in patients with polycythemia vera was independent from the presence of activated protein C resistance. Leiden mutation was observed in only 1 patient out of 4 patients with chronic myelogenous leukemia who had activated protein C resistance, but not thrombosis. Factor VIII levels of patients with chronic myelogenous leukemia (158% ± 14) were higher than healthy control subjects (99% ± 15) ($p < 0.05$). Patients with activated protein C resistance in both groups had no seropositivity for anticardiolipin antibodies IgG and IgM. Activated protein C resistance and in some cases its association with Leiden mutation in polycythemia vera may not have a major role in the pathogenesis of thromboembolic complications of polycythemia vera.

Key Words: Activated protein C resistance, Polycythemia vera, Chronic myelogenous leukemia.

INTRODUCTION

Primary role of hemostatic mechanisms that triggers thrombosis have not yet fully understood in various disorders associated with thromboembolic complications. Hypercoagulability can be broadly defined as a risk of thrombosis in circumstances that would not cause thrombosis in a normal subject.

Laboratory tests used in the determination of tendency for thrombosis are not sufficient for diagnosis. Spesific hemostatic defects (deficiencies of antithrombin III (ATIII), protein C (PC), protein S (PS) and plasminogen deficiency, etc.) can be identified in approxiimately 20% of patients with thrombotic disorders.

A defect in anticoagulant response to activated PC (APC), termed APC resistance (APCR), is a major risk factor for thrombosis, and is about 5-10 times more frequent than specific hemostatic defects[1-4]. The mode of inheritance in APCR is autosomal dominant. Factor V (FV) can not function as a natural anticoagulant in APC system, but may serve as a procoagulant in the formation of thrombin due to a molecular defect in factor V Leiden[5]. A single point mutation in FV gene (G to A substitution at codon 1691) results in mutant FV being called FV Leiden that interrupts inactivation of FV by APC and causes APCR[6,7]. Consequently, reduced sensitivity of mutant FV to APC mediated degradation results in thrombin formation.

Polycythemia vera (PV) is a haematologic malignancy that leads to excessive proliferation of erythroid, myeloid and megakaryocytic elements within the bone marrow. Hemorrhage is present in 15-35% of cases and responsible for 6-30% of deaths[8]. Cardiovascular events (venous thromboses, coronary thrombosis, claudication, acroparesthesia, the raynaud phenomenon, thromboangiitis obliterans) are more common (30-67%) than hemorrhages[9-11]. Splanchnic venous thromboses are observed in the half of the patients with thrombosis[11]. The number of platelets is frequently increased (80%), and morphological and qualitative functional platelet abnormalities are often detectable. Also, increased platelet activator inhibitor 1 levels, deficiencies of natural anticoagu-

lants and APCR have been described[11-16].

The aim of this study is to evaluate the role APCR in the hypercoagulable state seen in PV. Results of patients with PV (n: 24) were compared with the those of patients with chronic myelogenous leukemia (CML) (n: 27) and healthy control group (n: 52), because APCR was observed in some patients with myeloproliferative diseases (PV), essential thrombocytosis (ET), but these patients were not compared with such a myeloproliferative disease (CML) in which increased risk of thromboembolism was not observed.

MATERIALS and METHODS

Twenty-four patients with PV and a control group of 27 patients with CML and 52 healthy subjects were enrolled in this study from Haematology departments of Gülhane Military Medical Academy Haydarpa¾a Training Hospital and Marmara Medical Faculty. Exclusion criteria for the patients were the use of drugs that may affect haemostatic parameters, pregnancy, chronic liver disease or renal failure and active infection. In the control group, subjects with a history of thromboembolic accident and the use of drugs that may affect parameters of coagulation were excluded.

Patients were evaluated for the duration of the disease, history of thromboembolic accident and the given medication. Biochemical blood analysis (erythrocyte sedimentation rate, glycemia, urea, creatinine, uric acid, electrolytes, aspartate aminotransferase, alanine aminotransferase, alkaline phosphotase, lactate dehydrogenase, g-glutamyl transferase, bilirubins, cholesterole, tryglycerides, total protein, albumin) and haematologic parameters (whole blood count, bleeding and clotting time, fibrinogen, prothrombine time) were routinely performed.

Diagnostic procedures for deep vein thrombosis (DVT) included Doppler ultrasonography; for cerebrovascular thrombosis cranial computed tomography, magnetic resonance imaging or angiography and for ischemic heart disease (IHD) electrocardiography or coronary angiography.

Whole blood count was performed with Coulter MD II and routine biochemical parameters with

R-A 1000 and RA-XT (Technicon) autoanalyzer.

Venous blood samples for fibrinogen levels and prothrombin time were collected from an antecubital vein, anticoagulated with citrate (1 mL of 3.8% sodium citrate and 9 mL of venous blood), centrifugated at 1000 g for 20 minutes, and studied in 2 hours. IL Test PT-Fibrinogen HS Half Volume kit (Instrumentation Laboratory Company-Lexington, MA02173-3190, USA) with a serial number of 261044 was used. Calculations were performed on ACL 200 coagulometer (nephelometric centrifugal analyzer) (ACL Coagulation Systems. Instrumentation Laboratory SpA-Viale Monza 33-20/28, Milano, Italy) with the addition of calcium thromboplastin. Normal plasma values for fibrinogen is 2.00-4.00 gm/L and prothrombin time 10.7-13.0 seconds.

APCR test was performed on both patients and healthy subjects. APCR ratio was evaluated by a commercial method using an aPTT based Coatest APC resistance kit (Chromogenix AB, Taljegardsgatan 3, S-431 53 Mölndal, Sweden) with a serial number of 82 26 43-63/8. Nine mL of venous blood anticoagulated with 1 mL 0.1 mol/L of sodium citrate, centrifugated at 2000 g for 20 minutes. Plasma samples were stored at -70°C for 1month or studied in a 4-hour period. Frozen plasma incubated at 37°C, and APC/CaCl$_2$ and CaCl$_2$ solution heated. 100 microliters of plasma with 100 microliters of aPTT solution incubated for 5 minutes. The anticoagulant effect of APC was estimated as a ratio of recalcification times with or without APC on ACL 200 coagulometer. APC-ratio was calculated as follows:

APC-ratio= Clotting time (APC/CaCl$_2$)/ Clotting time (CaCl$_2$)

APC-ratios below the "cut-off" values were studied for a second time. An APC-ratio less than 1.37 (mean-2 standard deviation of healthy control group) defined as APCR.

Factor VIII (FVIII) activity was determined by an aPTT assay (Immuno-depleted Plasma for Factor VIII: C Assay by STA, Cat. No. 00725) using severe haemophilia A plasma as substrate and an automatic clot timer. The assay measures the ability of exogenously added FVIII to shorten FVIII deficient plasma; comparison with normal pooled plasma (standard 100% activity) enables quantitation of the percentage of activity. Collected bloods (9 vol.) were anticoagulated (1 vol. Trisodium citrate 3.2%), centrifugated at 2000 g for 15 minutes, stored in plastic tubes at -80°C for 1 month and studied in a 2-hour period. FVIII levels between 60-150% was considered as normal range.

Patients with APCR were studied for cardiolipin antibodies (ACA IgG and IgM) by ELISA method.

Subjects with APCR underwent analysis for the Leiden mutation. The 224 basepair fragment of the FV gene from patients with APCR was amplified with PCR as previously described[17]. Digestion of the PCR product with Mn/L resulted in 3 fragments of 104, 83 and 37 basepair in the normal gene and, 141 and 37 basepair in the mutant gene carrying AÆG mutation at position 1691.

Values are given as mean ± SEM. For conventional statistics, Mann-Whitney U and a chi-square test were used. When low expected values were found, a Fisher's exact test was used for further analyses. Differences in which $p < 0.05$ were regarded as significant.

RESULTS

Characteristics of the patients and the controls, including age, sex, duration of the disease, whole blood count and fibrinogen levels are shown in Table 1.

FVIII levels, ACA IgG and IgM, APCR and Leiden mutation of the patients and the control group are shown in Table 2.

Venous thromboembolism (VTE) was observed in 3 patients (12.5%) with PV, but none in CML group. Arterial thromboembolism was seen in 10 patients with PV (41.6%) and 2 patients (7.4%) with CML. Thromboembolic complications of the patients with PV were 5 ischemic heart diseases (IHD), 3 cerebrovascular accidents (CVA), 2 IHD + CVAs, 2 deep vein thrombosis (DVT) + CVAs and one DVT. Two patients with CML had IHD.

Table 1. Characteristics of the patients and the control group

	Control	PV	CML	p
Age (mean years ± SD)	62 ± 2.1	61 ± 2.2	64 ± 2.5	p> 0.05
Female/Male	25/27	11/13	12/15	p> 0.05
Duration of Disease (mean years ± SD)	-	3.7 ± 2.4	2.6 ± 1.7	-
White blood cells x 10^9/L	7.3 ± 0.2	13.9 ± 2.5	17.1 ± 4.7	p< 0.05
Haemoglobin (gr/L)	130 ± 8	155 ± 4	112 ± 5	p< 0.01
Platelet count (10^9/L)	240 ± 8	369 ± 39	307 ± 69	p< 0.01*
Fibrinogen (gm/L)	2.85 ± 0.07	3.83 ± 0.17	3.73 ± 0.23	p< 0.01

Values are given as mean ± 1SEM. Comparisons are between the patients with PV and CML and the control group.
* Significant difference only for PV group.

Patients with PV had higher haemoglobin values (155 ± 4 g/L) than the control group (130 ± 8 g/L) (p< 0.01) and this was independent from the risk of thromboembolism (p> 0.05). Haemoglobin values of the patients with CML (112 ± 5 g/L) were lower than the control group (p< 0.01).

Platelet count of the patients with PV (369 ± 39 10^9 /L) was higher than both CML (307 ± 69 10^9 /L) and control group (240 ± 8/10^9 /L) (p< 0.01). Difference between the CML and control group was not significant (p> 0.05). Presence or absence of thrombocytosis in patients with PV was not correlated with the observed thromboembolic complications (p> 0.05).

Fibrinogen levels were higher in both PV (3.83 ± 0.17 g/L) (p< 0.01) and CML group (3.73 ± 0.23 g/L) (p< 0.01) than the control group (2.85 ± 0.07 g/L). Difference between the patients with PV and CML were not significant (p> 0.05). There were not any significant relation between the elevated levels of fibrinogen and the risk of thromboembolism (p> 0.05).

APC-ratio was 2.91 ± 0.77 in the control group, so that an APC-ratio less than 1.37 (< mean-2 (SD)) defined as APCR. There was no significant difference in the APC-ratios between the PV (2.39 ± 0.86) and CML group (2.23 ± 0.62) (p> 0.05) (Figure 1). APCR was observed in 5 patients (20.8%) out of 24 patients with PV, 4 (14.8%) out of 27 patients with CML and one patient (1.9%) out of 52 control subjects. APCR was much more prevalent in the patient group than healthy control group (p< 0.05).

In patients with PV who had APCR; 2 patients had CVA + IHD, one DVT and one IHD. In the patient with DVT Leiden mutation was observed. One patient out of CML group who had APCR had IHD. Leiden mutation was observed in one patient who had APCR, but no thromboembolic complica-

Table 2. Parameters of the patients and the control group

	Control	PV	CML	p
FVIII (%)	99 ± 15	134 ± 19	158 ± 14	p< 0.05*
ACA IgG and IgM	-	0/5	0/4	-
APC-RATÝO	2.91 ± 0.77	2.39 ± 0.86	2.23 ± 0.62	p< 0.05
APCR	1/52	5/24	4/27	p< 0.05
Leiden mutation	0/1	1/5	1/4	-

Values are given as mean ± 1SEM. Comparisons are between the patients with PV and CML and the control group.
* Significant difference only for CML group.

tion was detected. There was no significant diffe-
rence in thromboembolic complications in patients
with PV according to the presence or absence of
APCR (p> 0.05). FVIII levels were higher in CML
group than the control group (p< 0.05), but not in
PV group (p> 0.05) (Figure 2). Patients with
APCR were studied for ACA IgG and IgM. ACA
IgG and IgM seropositivity was not observed in
any patient although 3 patients in PV group and 2
in CML had thromboembolic complications. Le-
iden mutation was observed in only one patient
from the each group who had APCR.

DISCUSSION

Thromboembolic complications in PV are
common, and in many instances, are the cause of
death[9,11]. Arterial and venous thromboses are
seen in 41-67% of the patients [10,18]. Cerebrovas-
cular system and portal vein district are common
site of thromboembolism[11,16,19]. Postoperative
thrombosis risk is also reported to be high (83%)
in patients with PV[20].

In our series of patients with PV, arterial
thrombosis (50% IHD, 30% CVA and 20% IHD +
CVA) was found in 41.6% and venous thrombosis
(DVT) in 12.5% of the patients.

Koster et al reported that subjects (with a first
episode of DVT, aged less than 70 years) with a
plasma fibrinogen greater than 5 g/L had an al-
most 4-fold increase of DVT and neither factor VII
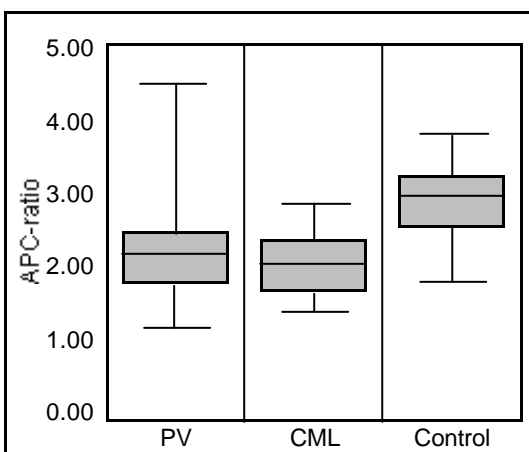and factor XII levels related to DVT[21,22].
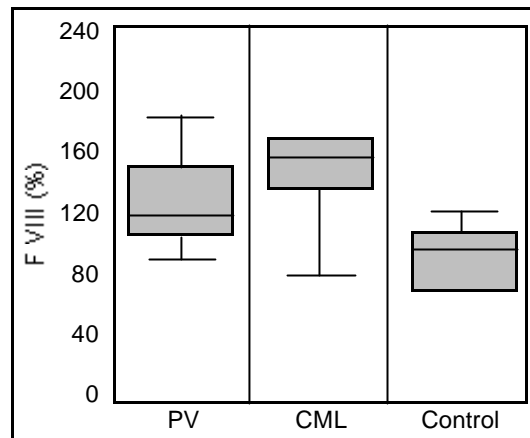

Figure 1. APC-ratio.

Figure 2. FVIII levels.

In our patients with PV and CML fibrinogen le-
vels were higher than in control subjects, but ele-
vated fibrinogen levels were not related with inc-
reased risk of thrombosis in patients with PV. Al-
so, increased haemoglobin levels and platelet co-
unts were not related with thrombosis risk in pati-
ents with PV.

Dahlback described that; APCR is found in
40% of the patients with unexplained thrombophi-
lia and in more than 90% of the cases caused by
Leiden mutation[23,24]. Simioni et al showed that in
patients with Leiden mutation thrombosis can oc-
cur in 42.3% of heterozygous cases and in 63%
homozygous cases[25]. Zoller et al also reported
that venous thrombosis is also found in 20% of
heterozygotes and 40% of homozygotes[26]. Me-
lichart et al concluded that in patients with venous
thrombosis and/or pulmonary embolism, APCR
was found in 26% of patients and the family his-
tory was positive in 60% of patients[27].

Prevalence studies from England and the Net-
herlands indicate that 1.7-3.5% and 1.7-2.0% of
the population, respectively, carries Leiden muta-
tion[28,29]. Özbek et al found a carrier rate of
9.16% and an allele frequency of 4.58% in Turkish
individuals[30].

In our healthy control group, APCR is found in
1.9% of cases, but none of these cases was as-
sociated with FV Leiden.

Bokarewa et al reported that APCR was also
present in 49% of cases with antiphospholipid an-

tibodies although, no correlation was observed between APCR and ACA, lupus anticoagulant activity and FV Leiden[31]. Griffin et al suggested that cardiolipin promoted APC anticoagulant activity better than activated factor X coagulant activity, and antibodies from some antiphospholipid antibody syndrome patients downregulated APC activity[32]. Bokarewa et al also observed APCR in 33% and both APCR and phospholipid antibodies in 22% of the women with a history of thromboembolism and, concluded that APCR was commonly triggered by endogenous (pregnancy, delivery) factors[33].

In our patients with PV and CML ACA IgG and IgM seropositivity were not observed.

Although, morphological and qualitative functional platelet abnormalities, thrombocytosis, erythrocytosis, high volume of packed red cells, low plasma volume and a great viscosity of the blood are suggested abnormalities in the thromboembolic complications of PV[15,16]. In our patients with PV thrombocytosis, leukocytosis and erythrocytosis were not related with thromboembolic complications.

Calcelas et al studied platelet count, AT III, PC, PS, von Willebrand's factor, tissue plasminogen activator and plasminogen activator inhibitor 1(PAI-1) levels in patients with ET and PV. They showed that levels of PAI-1 was increased and, correlated linearly with platelet counts and venous thromboembolism[13].

Teofili et al screened coagulative abnormalities associated with thrombophilia in patients with PV and ET. In 11.6% of patients (suffering from venous thrombosis prior to 45 years) a plasmatic thrombogenic defect (quantitative deficiency of AT III, PC, PS and plasminogen) was found[11].

In a study of Wieczorek et al, reduced levels of PC and PS (in addition to erythrocytosis, thrombocytosis and abnormal platelet functions) were found in patients with PV and they suggested that it may be secondary to chronic occult thrombosis[34].

Bucalossi et al evaluated natural anticoagulants and APCR in patients with PV. 63.6% of 33 patients showed thrombotic episodes. APCR was

evaluated in 16 cases of PV and 3 out of 20 patients with thrombosis had APCR (11 patients without thrombosis had no APCR). Natural anticoagulant deficit (AT III, PC, PS) was observed in 47.6% of patients with PV who have thrombosis, but in patients without thrombosis no deficit was found. Natural anticoagulant deficits in patients with thrombosis were not related with heredity, acquired deficiencies due to chemotherapy and subclinic disseminated intravascular coagulation. APCR in 3 cases supported the potential role of APCR in the pathogenesis of thrombosis. Natural anticoagulant deficits and/or APCR are thought to create a "prothrombotic" state in the multifactorial thrombosis pathogenesis of PV. Also, in this study no correlation was found between the risk of thrombosis and, platelet, leukocyte and erythrocyte counts[12,14].

Lamparter et al reported a patient with PV and recurrent thrombosis who had APCR and, concluded that the presence of APCR was an additional thrombotic risk factor[35].

In our study, thromboembolic episode was found in 13 cases in PV group. Five cases had APCR, and FV Leiden was observed in only one case with APCR. In PV patients with APCR 2 cases had CVA + IHD, 1 DVT and 1 IHD. The patient who had both APCR and FV Leiden suffered from DVT.

In our CML group 4 patients out of 27 (14.8%) had APCR. FV Leiden and also thrombotic episode were not identified in any of the patients who had APCR. Two cases with IHD had no APCR.

FVIII levels of the patients with CML were higher than that of healthy control group (Figure 2). This increased levels FVIII may be responsible from the increased APC-ratios. Also, Vasse and Mathonnet et al explained that an increase in FVIII level was associated with a decreased response to APC, when aPTT-based assays were used[36-38].

In patients with PV APCR was higher than that of control subjects. FV Leiden was partially responsible for the increased frequency of APCR. APCR in PV and its association with FV Leiden in

some patients supports the role of APCR in thromboembolic complications. In patients with CML, presence of APCR, but absence of thrombosis suggests that increased levels of FVIII may be associated with APCR.

In conclusion, APCR may be observed in patients with PV where it is associated, in some cases, with Leiden mutation, but it can not be concluded that APCR has a major role in the pathogenesis of thromboembolic complications of PV. Further studies are needed to elucidate the role of APCR associated with FV Leiden in the thromboembolic complications of PV.

REFERENCES

1. Dahlback B, Carlson 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 Nat Aca Sci USA 1993;90:1004.

2. Fainoi EM, Franchi F, Asti D, Sacchi E, Bernardi F, Manucci PM. Resistance to activated protein C in nine thrombophilic families: Interference in a protein S functional assay. Thromb Haemost 1993;70:1067.

3. Koster T. Leiden Thrombophilia Study: Venous thrombosis due to poor anticoagulant response to activated protein C. Lancet 1993;342:1530.

4. Bauer K. Hypercoagulability: a new cofactor in the protein C anticoagulant pathway. N Engl J Med 1994;330:566.

5. Tuddenham EGD. Thrombophilia: The new factor is old factor V. Lancet 1994;353:1515.

6. Bertina RM, Koeleman BP, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, van der Velden PA, Reitsma PH. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 1994;369:64.

7. Voorberg J, Roelse J, Koopman R. Association of idiopathic venous thromboembolism with single point mutation at arginin of factor V. Lancet 1994;343:1535.

8. William GH. Primary and secondary erythrocytosis. In: Joseph JM (ed). Manual of Clinical Hematology. Little Brown and Company, 1995;70.

9. Brown GE, Griffin HZ. Peripheral arterial disease in polycythemia vera. Arch Intern Med 1930;46:705.

10. Frezzato M, Rugger M, Castaman G, Rodeghiero F. Polycythemia vera and essential thrombocythemia in young patients. Haematologica 1993;78:11.

11. Teofili L, De Stefano V, Leone G, Micalizzi P, Lovi-no MS, Alfano G, Bizzi B. Hematological causes of venous thrombosis in young people: High incidence of myeloproliferative disorder as underlying disease in patients with splanchnic venous thrombosis. Thromb Haemost 1992;67:297.

12. Bucalossi A, Marotta G, Bigazzi C, Galieni P, Dispensa E. Reduction of antithrombin III, protein C and protein S levels and activated protein C resistanca in polychthemia vera and essential thrombocythemia patients with thrombosis. Am J Hematol 1996; 52:14.

13. Calcelas JA, Garcia AA, Garcia FLJ. High plasma levels of PAI-1 in polycythemia vera and essential thrombocythemia are associated with thrombosis. Thromb Res 1994;75:513.

14. Carvalho A, Ellman L. Activation of coagulation system in polycythemia vera. Blood 1976;47:669.

15. Grune & Stratton, Berlin NI. Diagnosis and classification of the polycythemias. Semin Haematol 1975;12:339.

16. John WA. Polycythemia vera. In: Wintrobe MM (ed). Wintrobe's Clinical Hematology. Philadelphia: Lea and Febiger, 1993, p 1999.

17. Ridker PM, Hennekens CH, Lindpaintner K, Stampfer MJ, Eisenberg PR. Mutation in the gene coding for coagulation factor V and the risk of myocardial infarction, stroke, and venous thrombosis in apparently healthy men. N Engl J Med 1994;332:912.

18. Gruppo Italiano Studio Policitemia Polycythemia Vera: The natural history of 1213 patients followed for 20 years. Ann Intern Med 1995;123:656.

19. Randi ML, Fabris F, Tison T, Barbone E, Rossi C, Girolami A. Prevalence of specific thrombotic accidents in patients with thrombocytosis. Haematologia 1993;25:149.

20. Wasserman LR. The management of polycythemia vera. Br J Haematol 1971;21:371.

21. Koster T, Rosendaal FR, Briet E, Vandenbroucke JP. John Hageman's factor and deep vein thrombosis: Leiden thrombophilia study. Br J Haematol 1994; 87:422.

22. Koster T, Rosendaal FR, Reitsma PH, van der Velden PA, Briet E, Vandenbroucke JP. Factor VII and fibrinogen levels as risk factors for venous thrombosis. A case-control study of plasma levels and DNA polymorphism-the Leiden Thrombophilia Study (LETS). Thromb Haemost 1994;71:719.

23. Dahlback B. Inherited resistance to activated protein C, a major cause of venous thrombosis, is due to a mutation in factor V gene. Haemostasis 1994;24:139.

24. Dahlback B. Factor V gene mutation causing inherited resistance to activated protein C as a basis for venous thromboembolism. J Intern Med 1995;237:21.

25. Simioni P, Scarano L, Gavasso S, Sardella C, Giro-

lami B, Scudeller A, Girolami A. Prothrombin fragment 1 + 2 and thrombin-antithrombin complex levels in patients with inherited APC resistance due to factor V Leiden mutation. Br J Haematol 1996;92: 435.

26. Zoller B, Svensson PJ, He X, Dahlback B. Identification of the same factor V gene mutation in 47 out of 50 thrombosis-prone families with inherited resistance to activated protein C. J Clin Invest 1994;94: 2521.

27. Melichart M, Kyrle PA, Eichinger S, Rintelen C, Mannhalter C, Pabinger I. Thrombotic tendency in 75 symptomatic unrelated patients with APC resistance. Wien Klin Wochenschr 1996;108:607.

28. Beauchamp NJ, Daly ME, Hampton KK, Cooper PC, Preston FE, Peake IR. High prevalence of a mutation in the factor V gene within the UK population: Relationship to activated protein C resistance and familial thrombosis. Br J Haematol 1994;88: 219.

29. Bertina RM, Koeleman BP, Koster T, Rosendaal FR, Dirven RJ, de ronde H, van der Velden PA, Reitsma PH. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 1994;369:64.

30. Özbek U, Tangün Y. Frequency of factor V Leiden in Turkey. Int J Hematol 1996;64(3-4):291.

31. Bokarewa MI, Blomback M, Bremme K. Phospholipid antibodies and resistance to activated protein C in women with thrombophilia. Blood Coagul Fibrinolysis 1995;6:417.

32. Griffin JH, Heeb MJ, Kojima Y, Fernandez JA, Kojima K, Hackeng TM, Greengard JS. Activated protein C resistance: Molecular mechanisms. Thromb Haemost 1995;74:444.

33. Bokarewa MI, Bremme K, Falk G, Sten LM, Egberg N, Blomback M. Studies on phospholipid antibodies, APC-resistance and associated mutation of factor V gene. Thromb Res 1995;78:193.

34. Wieozorek I, MacGregor IR, Ludlam CA. Low protein C and S and activation of fibrinolysis in treated essential trombocythemia. Am J Hematol 1995;49: 277.

35. Lamparter S, Schuermann M, Heidtmann HH. APC resistance as an additional thrombotic risk factor in a patient suffering from polycythemia vera and recurrent thrombosis. Ann Hematol 1997;74:49.

36. Vasse M, Leduc O, Borg JY, Chretien MH, Monconduit M. Resistance to activated protein C: Evaluation of three functional assays. Thromb Res 1994;76: 47.

37. Mathonnet F, de Mazancourt P, Bastenaire B, Morot M, Benattar N, Beufe S, Borg JY, Giudicelli Y. Activated protein C sensitivity ratio in pregnant women at delivery. Br J Haematol 1996;92:244.

38. Mathonnet F, de Mazancourt P, Denninger MH, Morot M, Benattar N, Giudicelli Y. Role of factor VIII on activated protein C resistance ratio in inflammatory diseases. Br J Haematol 1996;95:423.

Address for Correspondence:

Ahmet ÖZTÜRK, MD

Koþuyolu Hava Lojmanlarý D Blok No:3
Üsküdar, Ýstanbul, TURKEY