

Soluble Platelet Glycoprotein V in Distinct Disease States of Pathological Thrombopoiesis

Kadir Acar, MD; Salih Aksu, MD; Yavuz Beyazit, MD; Ibrahim C. Haznedaroglu, MD; Ebru Koca, MD; Deniz Cetiner, MD; Nilgün Sayinalp, MD; Yahya Buyukasik, MD; Hakan Goker, MD; Serafettin Kirazli, PhD; and Osman I. Ozcebe, MD

Quantitative platelet disorders (i.e., thrombocytosis or thrombocytopenia) may also be associated with qualitative platelet alterations. Clonal thrombocythemia (CT), reactive thrombocytosis (RT), immune thrombocytopenic purpura (ITP), and thrombocytopenia of aplastic pancytopenia (AA) or infiltrative bone marrow disorders represent the major classes of pathological thrombopoiesis. Glycoprotein V may serve as an *in vivo* marker of platelet activation in thrombotic and hemorrhagic states. The aim of this study was to assess circulating plasma soluble platelet glycoprotein V (sGPV) concentrations in distinct disease states of pathological thrombopoiesis. The whole study group comprised 20 patients with thrombocytopenia, 32 patients with thrombocytosis and 14 healthy adults as the control group. sGPV was significantly increased in the group of thrombocytosis patients in comparison to the thrombocytopenic group and the healthy control groups. When sGPV levels were corrected according to platelet number (sGPV/tr), this ratio was very high in patients with thrombocytopenia compared to patients with thrombocytosis and the control group. Our results suggest that there is an ongoing platelet activation associated with thrombocytosis regardless of its origin is either CT or RT. Therefore, glycoprotein V system may serve to activate residual platelets in thrombocytopenia regardless of its origin is either ITP or AA.

Key words: platelets, thrombocytopenia

© 2008. From the Department of Hematology, Selcuk University Meram Medical School, Konya, Turkey (Acar); and the Department of Internal Medicine and Hematology, Hacettepe University Medical School (Aksu, Beyazit, Haznedaroglu, Koca, Cetiner, Sayinalp, Buyukasik, Goker, Kirazli, Ozcebe), Ankara, Turkey. Send correspondence and reprint requests for *J Natl Med Assoc.* 2008;100:86-90 to: Dr. Kadir Acar, Selcuk University Meram Medical School, Department of Hematology, 42001 Meram, Konya, Turkey; phone: +90-332-223-69-11; fax: +90-332-223-6184; e-mail: acarkadir@yahoo.com

INTRODUCTION

A wide variety of quantitative platelet disorders presenting with either thrombocytopenia or thrombocytosis in many distinct disease states may also be associated with qualitative platelet alterations even after the normalization of platelet counts.¹⁻⁴

Clonal thrombocythemia (CT) of myeloproliferative diseases is usually associated with qualitative platelet abnormalities, sometimes leading to clinical thrombosis and/or bleeding.^{1,5,6} On the other hand, reactive thrombocytosis (RT) is a relatively benign laboratory secondary abnormality, which is usually not associated with qualitative platelet defects.^{5,7-10} Immune thrombocytopenic purpura (ITP) is a unique form of thrombocytopenia with increased residual platelet procoagulant activity.^{2,3,5,7,11-17} However, thrombocytopenia of aplastic pancytopenia (AA) or infiltrative bone marrow disorders does not have such compensatory enhanced platelet function, which makes the patient more prone to bleeds.^{3,5,7,12,18-21} Many cytokines, growth factors, hormones, proteins and molecules have been searched for the clarification of qualitative platelet alterations in quantitative platelet disorders,^{1-4,7,8,10,14,16,17,19,22-27} although the exact mechanisms remained to be elucidated.

Glycoprotein V is an important subunit of the platelet membrane glycoprotein Ib-V-IX receptor for von Willibrand factor and thrombin. Glycoprotein V is cleaved from the platelet surface during activation by the thrombin.²⁸⁻³⁴ Thus, the molecule may serve as an *in vivo* marker of platelet activation in thrombotic states like other platelet activation markers.^{2,35-37} The aim of this study is to assess circulating plasma-soluble glycoprotein-V (sGPV) concentrations in distinct disease states of pathological thrombopoiesis. Patients with CT, RT, ITP or AA have been included to represent the subpopulations of pathological platelet formation. The hypothesis was that the sGPV reflects ongoing platelet activation during the complicated clinical course of those quantitative platelet disorders. Elucidation of the importance of the glycoprotein-V system in pathological thrombopoiesis will not only help better understanding of those enigmatic states but may also aid better vascular management of the diseases associated with quantitative platelet abnormalities to prevent both bleeding and thrombosis.

PATIENTS AND METHODS

The whole study group comprised 20 patients with thrombocytopenia, 32 patients with thrombocytosis and

14 healthy adults as the control group. Patients with thrombocytopenia consisted of patients who are thrombocytopenic due to either accelerated peripheral platelet destruction [13 patients (five males and eight females, median age 30 years) with ITP], or impaired platelet production of aplastic bone marrow (AA) (five patients with clonal aplastic anemia, one with Fanconi aplastic anemia, one patient who received marrow suppressive chemotherapy for cervical carcinoma; comprising three males and four females, median age 58 years). Median platelet count in the ITP group was 27,000/mm³ (5,000–96000/mm³), whereas platelet counts were 22,000/mm³ (11,000–86,000/mm³) in the AA group. The group with thrombocytosis consisted of patients with CT or RT. The group with CT included patients with chronic myeloproliferative diseases (10 patients with essential thrombocythemia (ET), six with chronic myelogenous leukemia (CML), three with polycythemia vera (PV); comprising eight males and 11 females; median age 56 years). Median platelet number in the CT group was 889,000/mm³ (496,000–2,671,000/mm³). Patients with RT had a disease known to be associated with secondary thrombocytosis (four infection, four malignancy, four inflammatory disease, one iron deficiency anemia; comprising five males and eight females; median age 39 years). Median platelet number in the RT group was 737,000/mm³ (455,000–1,060,000/mm³). The control group consisted of 14 healthy adults (10 males and four females aged 45 years). Median platelet number of the healthy controls was 263,000/mm³ (169,000–3,980,000/mm³).

Fasting blood was collected from the large peripheral veins of the studied subjects without using a tourniquet. The first 2 mL of blood samples were discarded, then 4.5 ml of blood were taken into Diatube H tubes (Becton Dickinson, Plymouth, UK). sGPV levels were determined by the commercially available enzyme immunoassay (ELISA) method by using the Asserachrom Soluble GPV immunoassay (Diagnostica Stago, Asnières, France). The experiment was performed

according to the suggestions of the manufacturer. Standards and samples were pipetted into the wells pre-coated with the first monoclonal antibody specific for GPV. A second monoclonal antibody directed against another epitope of the sGPV, coupled with peroxidase was added after washing. Following the washing process, a substrate solution was added, and the color developed in proportion to the amount of GPV bound in the initial step. The color development was stopped, and the intensity of the color was measured at the last step.

Results were expressed as median and interquartile (25–75th percentile) range. Nonparametric tests were used to compare continuous values between groups (Mann-Whitney or Kruskal-Wallis tests, depending on the number of groups). To calculate sGPV per thrombocyte, the level of sGPV was divided into the platelet count. All significant levels were set at 0.05. Computations have been run with the package program of SPSS v. 12.0 (SPSS Inc).

RESULTS

Plasma levels of sGPV were significantly increased in the group of thrombocytosis patients in comparison to the thrombocytopenic group ($p < 0.0001$) and the healthy control groups ($p < 0.0001$) (Table 1). Although sGPV levels between the subtypes of thrombocytosis ($p = 0.012$) patients were significantly different, sGPV levels between subtypes of thrombocytopenia ($p = 0.913$) patients were not differ significantly. When GPV levels were corrected according to platelet number (sGPV/tr), this ratio was very high in patients with thrombocytopenia compared to patients with thrombocytosis ($p < 0.001$) and the control group ($p < 0.001$). sGPV/tr ratio was not significantly different between patients with thrombocytosis and the control group ($p = 0.057$). sGPV/tr levels between the subtypes of thrombocytosis ($p = 0.842$) and thrombocytopenia ($p = 0.877$) patients did not differ significantly (Figures 1 and 2).

DISCUSSION

In this study, peripheral circulating blood sGPV lev-

Table 1. Median (IQR) soluble platelet glycoprotein V (sGPV) in distinct disease states of pathological thrombopoiesis (clonal thrombocythemia, reactive thrombocytosis, aplastic thrombocytopenia, immune thrombocytopenia and healthy adults as controls)

Patients (n)	sGPV (ng/ml)	sGPV/Platelet
Thrombocytosis		
Clonal (19)	209.0 (80.5–657.0) ^{a,b}	0.21 (0.094–2.350) ^d
Reactive (13)	127.0 (36.6–500.0) ^{a,b}	0.19 (0.061–1.330) ^d
Total (32)	158.8 (36.6–657.0) ^{a,b}	0.20 (0.061–2.350) ^d
Thrombocytopenia		
Aplasia (7)	42.2 (28.6–75.2) ^c	1.83 (0.220–4.70) ^e
Immune (13)	40.4 (19.9–241.0) ^c	1.43 (0.570–5.770) ^e
Total (20)	41.2 (19.9–241.0) ^c	1.49 (0.220–5.770) ^e
Healthy Controls		
Control (14)	81.8 (45.4–111.0)	0.34 (0.193–0.441)

a: $P < 0.01$ versus controls; b: $P < 0.01$ versus thrombocytopenic patients; c: $P < 0.01$ versus controls; d: $P < 0.01$ versus thrombocytopenic patients; e: $P < 0.01$ versus controls

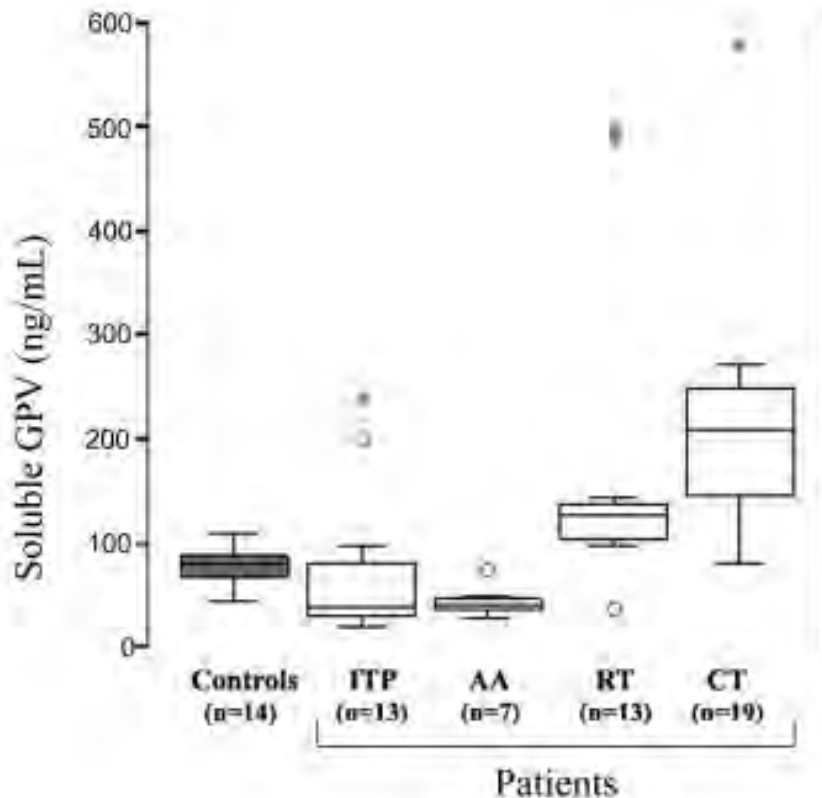
els were significantly higher in thrombocytosis in comparison to the thrombocytopenia and normal platelet counts (Figure 1). Glycoprotein V is a subunit of the GPIb-IX-V receptor for von Willebrand factor and thrombin and modulates platelet responses to the physiological platelet agonists such as thrombin and collagen. Agonist-induced platelet activation directly cleaves GPV from the platelet surface.³⁸ Our results demonstrating the increments in the circulating sGPV suggest that there is an ongoing platelet activation associated with thrombocytosis regardless of whether its origin is either CT or RT. Platelet activation, vascular dysfunction and coagulopathy are the critical pathobiological events in the genesis of thrombotic and hemorrhagic vascular complications of thrombocytosis.^{1,6,39-41} Although sGPV concentrations tended to be more elevated in CT than RT, that difference failed to reach a statistically significant level (Table 1). Bleeding and thrombosis in myeloproliferative disorders associated with platelet activation are common events; sometimes both are present in the same patient during the course of the disease.^{6,42} The presence of circulating activated platelets, using simultaneous flow cytometry and aggregometric, was observed in myeloproliferative disorders, as well as the

activation markers of endothelium and platelets.^{42,43} Glycoprotein V seems to precipitate platelet hyperfunctioning of thrombocythemia during the clinical course of distinct disorders.

Our results indicate that increments in platelet numbers are associated with increased sGPV concentrations and, thus, activated platelets. When GPV levels were corrected according to platelet number (sGPV/tr), this ratio was very high in patients with thrombocytopenia compared to patients with thrombocytosis and the control group (Figure 2). Therefore, the glycoprotein-V system may serve to activate residual platelets in thrombocytopenia regardless of whether its origin is ITP or AA. The probability of the occurrence and severity of bleeding complications differ significantly between individual patients with thrombocytopenia. Residual platelet activation, vascular dysfunction, infection and concurrent coagulopathy are also the critical pathobiological events in the genesis hemorrhagic vascular complications of thrombocytopenia.

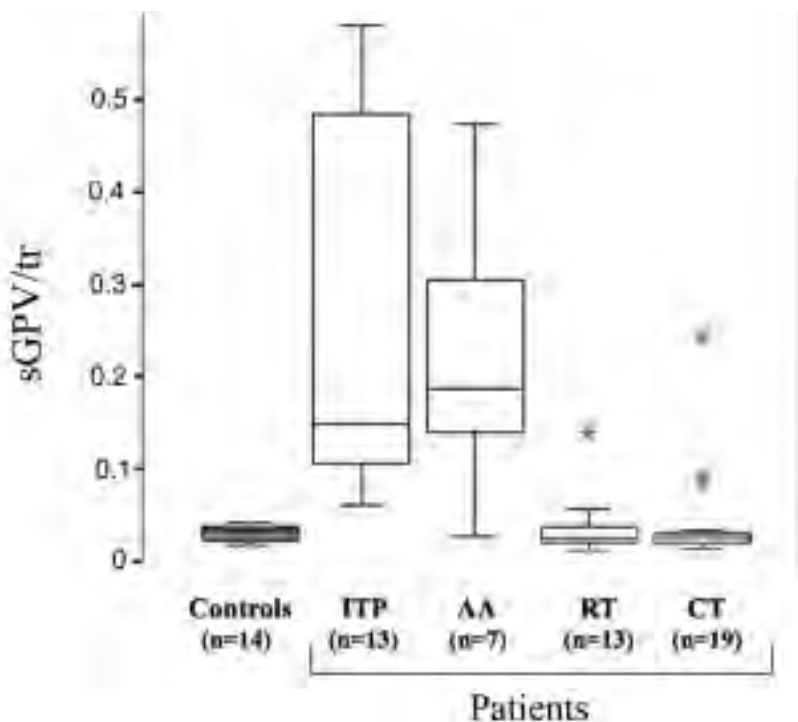
Platelet adhesion to an injured blood vessel wall is a critical initiating step in hemostasis mediated by a four-member receptor complex (glycoprotein Ib/V/IX) interacting with plasma von Willebrand factor. The function of the glycoprotein-V subunit plays a role within

Figure 1. Soluble platelet glycoprotein V (sGPV) levels in distinct disease states of pathological thrombopoiesis (clonal thrombocythemia, reactive thrombocytosis, aplastic thrombocytopenia, immune thrombocytopenia and healthy adults as controls



CT: clonal thrombocythemia, RT: Reactive thrombocytosis, AA: Aplastic thrombocytopenia, ITP: Immune thrombocytopenia

Figure 2. Soluble glycoprotein V (sGPV) levels that corrected according to platelet number (sGPV/tr) in distinct disease states of pathological thrombopoiesis (clonal thrombocythemia, reactive thrombocytosis, aplastic thrombocytopenia, immune thrombocytopenia and healthy adults as controls



CT: clonal thrombocythemia, RT: Reactive thrombocytosis, AA: Aplastic thrombocytopenia, ITP: Immune thrombocytopenia

the GPIIb/IIIa receptor complex by enhancing Ib alpha surface expression.⁴⁴ According to the results of our present study, the glycoprotein-V system acts as a compensatory system for the activation of platelets in thrombocytopenic states. That activation may be useful for preventing bleeding in patients with thrombocytopenia of any origin. On the other hand, however, glycoprotein V functions as a pathological additional risk factor for thrombotic complications in thrombocytosis. In vivo thrombin activity is extremely important in arterial occlusion. The glycoprotein Ib-IX-V complex contains a high-affinity thrombin-binding site on the platelet surface with a defined role in platelet activation by the thrombin. Thrombin and glycoprotein V have important interactions. Platelet surface expression of glycoprotein V is regulated by thrombin, thrombin receptor-activating peptide and a cytoskeletal-mediated redistribution of platelet surface GPV to the surface-connected canalicular system. Thrombin proteolyzes platelet surface glycoprotein V. Platelet glycoprotein V also binds to collagen and participates in platelet adhesion and aggregation.^{32,34,45-50} Increments in the sGPV concentrations in thrombocytosis seem to be relating to pathological platelet activation of the disease course in association with receptor-ligand cross-talks. Further clinical and experimental studies are needed to determine complicated cross-talks of the platelet glycoprotein receptors and their ligands, especially for the

better management of patients with pathological platelet formation.

REFERENCES

- Blann A, Caine G, Bareford D. Abnormal vascular, platelet and coagulation markers in primary thrombocythaemia are not reversed by treatments that reduce the platelet count. *Platelets*. 2004;15:447-449.
- Haznedaroglu IC, Buyukasik Y, Kosar A, et al. Thrombopoietin, interleukin-6, and P-selectin at diagnosis and during post-steroid recovery period of patients with autoimmune thrombocytopenic purpura. *Ann Hematol*. 1998;77:165-170.
- Kosar A, Haznedaroglu IC, Buyukasik Y, et al. Circulating thrombopoietin and interleukin-6 in newly diagnosed autoimmune versus aplastic thrombocytopenia. *Haematologica*. 1998;83:1055-1056.
- Haznedaroglu IC, Ertenli I, Ozcebe OI, et al. Megakaryocyte-related interleukins in reactive thrombocytosis versus autonomous thrombocythemia. *Acta Haematol*. 1996;95:107-111.
- Haznedaroglu IC, Gullu IH, Dundar S, et al. The significance and distinct interactions of various growth factors in physiologic and pathologic megakaryocytopoiesis/thrombocytopoiesis. *Aust N Z J Med*. 1997;27:191-192.
- Bas B, Koksak A, Ozatli D, et al. Thrombosis and hemorrhage in chronic myeloproliferative disorders. *Clin Appl Thromb Hemost*. 1999;5:282-284.
- Cobankara V, Oran B, Ozatli D, et al. Cytokines, endothelium, and adhesive molecules in pathologic thrombopoiesis. *Clin Appl Thromb Hemost*. 2001;7:126-130.
- Ertenli I, Kiraz S, Ozturk MA, et al. Pathologic thrombopoiesis of rheumatoid arthritis. *Rheumatol Int*. 2003;23:49-60.
- Ozturk MA, Kiraz S, Ertenli I, et al. Proinflammatory and hematopoietic cytokines in reactive and clonal thrombocytosis. *South Med J*. 2002;95:565-566.
- Ertenli I, Haznedaroglu IC, Kiraz S, et al. Cytokines affecting megakaryocytopoiesis in rheumatoid arthritis with thrombocytosis. *Rheumatol Int*. 1996;16:5-8.

11. Ahn YS, Jy W, Kolodny L, et al. Activated platelet aggregates in thrombotic thrombocytopenic purpura: decrease with plasma infusions and normalization in remission. *Br J Haematol*. 1996;95:408-415.
12. Haznedaroglu IC, Savas CM, Benekli M, et al. The significance of megakaryocytopoietic cytokines and thrombopoietin in immune thrombocytopenic purpura: a hypothesis. *N Z Med J*. 1996;109:389.
13. Nomura S, Kido H, Yamaguchi K, et al. Platelet activation by antiplatelet autoantibodies in immune thrombocytopenic purpura. *Eur J Haematol*. 1992;49:109-110.
14. Sayinalp N, Haznedaroglu IC, Buyukasik Y, et al. Protein C inhibitor and serum amyloid A in immune thrombocytopenic purpura. *J Int Med Res*. 2004;32:62-65.
15. Rand ML, Dean JA. Platelets function in autoimmune (idiopathic) thrombocytopenic purpura. *Acta Paediatr Suppl*. 1998;424:57-60.
16. Haznedaroglu IC, Sayinalp NM, Ozcebe OI, et al. Megakaryocytopoietic cytokines in autoimmune thrombocytopenic purpura. *Am J Hematol*. 1995;49:265.
17. Haznedaroglu IC, Buyukasik Y, Kosar A, et al. Selectins and IL-6 during the clinical course of idiopathic thrombocytopenic purpura. *Acta Haematol*. 1999;101:16-20.
18. Yildiz BO, Haznedaroglu IC, Coplu L. Albendazole-induced amegakaryocytic thrombocytopenic purpura. *Ann Pharmacother*. 1998;32:842.
19. Ozatli D, Kocoglu H, Haznedaroglu IC, et al. Circulating thrombomodulin, thrombospondin, and fibronectin in acute myeloblastic leukemias. *Haematologia (Budap)*. 1999;29:277-283.
20. Turgut M, Sunbul M, Bayirli D, et al. Thrombocytopenia complicating the clinical course of leptospiral infection. *J Int Med Res*. 2002;30:535-540.
21. Gursoy M, Haznedaroglu IC, Celik I, et al. Agranulocytosis, plasmacytosis, and thrombocytosis followed by a leukemoid reaction due to acute acetaminophen toxicity. *Ann Pharmacother*. 1996;30:762-765.
22. Haznedaroglu IC, Ozcebe OI, Dundar SV, et al. Haematopoietic cytokines and increased megakaryocytic proliferation in chromosome 5q deletion. *Acta Haematol*. 1997;97:244.
23. Asik M, Karakus S, Haznedaroglu IC, et al. Bone Marrow and Peripheral Blood C-kit Ligand Concentrations in Patients with Thrombocytosis and Thrombocytopenia. *Hematology*. 2003;8:369-373.
24. Haznedaroglu IC, Goker H, Turgut M, et al. Thrombopoietin as a drug: biologic expectations, clinical realities, and future directions. *Clin Appl Thromb Hemost*. 2002;8:193-212.
25. Karakus S, Ozcebe OI, Haznedaroglu IC, et al. Circulating thrombopoietin in clonal versus reactive thrombocytosis. *Hematology*. 2002;7:9-12.
26. Kiraz S, Ertenli I, Ozturk MA, et al. Bloodstream thrombopoietin in rheumatoid arthritis with thrombocytosis. *Clin Rheumatol*. 2002;21:453-456.
27. Sayinalp N, Haznedaroglu IC, Ozdemir O, et al. Interleukin-1 beta and interleukin-6 in clonal versus reactive thrombocytosis. *Eur J Haematol*. 1995;55:339-440.
28. Aleil B, Mossard JM, Wiesel ML, et al. Increased plasma levels of soluble platelet glycoprotein V in patients with acute myocardial infarction. *J Thromb Haemost*. 2003;1:1846-1847.
29. Ramakrishnan V, DeGuzman F, Bao M, et al. A thrombin receptor function for platelet glycoprotein Ib-IX unmasked by cleavage of glycoprotein V. *Proc Natl Acad Sci U S A*. 2001;98:1823-1828.
30. Zafar RS, Walz DA. Platelet membrane glycoprotein V: characterization of the thrombin-sensitive glycoprotein from human platelets. *Thromb Res*. 1989;53:31-44.
31. Roth GJ, Church TA, McMullen BA, et al. Human platelet glycoprotein V: a surface leucine-rich glycoprotein related to adhesion. *Biochem Biophys Res Commun*. 1990;170:153-161.
32. Dong JF, Sae-Tung G, Lopez JA. Role of glycoprotein V in the formation of the platelet high-affinity thrombin-binding site. *Blood*. 1997;89:4355-4363.
33. Mayer JL, Beardsley DS. Varicella-associated thrombocytopenia: autoantibodies against platelet surface glycoprotein V. *Pediatr Res*. 1996;40:615-619.
34. Michelson AD, Benoit SE, Furman MI, et al. The platelet surface expression of glycoprotein V is regulated by two independent mechanisms: proteolysis and a reversible cytoskeletal-mediated redistribution to the surface-connected canalicular system. *Blood*. 1996;87:1396-1408.
35. Atilar E, Haznedaroglu I, Aytemir K, et al. Effects of stent coating on platelets and endothelial cells after intracoronary stent implantation. *Clin Cardiol*. 2001;24:159-164.
36. Buyukasik Y, Soyul B, Soyul AR, et al. In vivo platelet and T-lymphocyte activities during pulmonary tuberculosis. *Eur Respir J*. 1998;12:1375-1379.
37. Ozcebe OI, Karakus S, Haznedaroglu IC, et al. Plasma thrombospondin in immune thrombocytopenic purpura. *J Int Med Res*. 2002;30:52-55.
38. Rabie T, Strehl A, Ludwig A, et al. Evidence for a role of ADAM17 (TACE) in the regulation of platelet glycoprotein V. *J Biol Chem*. 2005;280:14462-14468.
39. Michiels JJ. Platelet-mediated microvascular inflammation and thrombosis in thrombocytopenia: a distinct aspirin-responsive arterial thrombophilia, which transforms into a bleeding diathesis at increasing platelet counts. *Pathol Biol (Paris)*. 2003;51:167-175.
40. Viillard JF, Solanilla A, Gauthier B, et al. Increased soluble and platelet-associated CD40 ligand in essential thrombocythemia and reactive thrombocytosis. *Blood*. 2002;99:2612-2614.
41. Villmow T, Kemkes-Matthes B, Matzdorff AC. Markers of platelet activation and platelet-leukocyte interaction in patients with myeloproliferative syndromes. *Thromb Res*. 2002;108:139-145.
42. Bermejo E, Alberto MF, Meschengieser SS, et al. Assessment of platelet activation in myeloproliferative disorders with complementary techniques. *Blood Coagul Fibrinolysis*. 2004;15:235-240.
43. Karakantza M, Giannakoulas NC, Zikos P, et al. Markers of endothelial and in vivo platelet activation in patients with essential thrombocythemia and polycythemia vera. *Int J Hematol*. 2004;79:253-259.
44. Calverley DC, Yagi M, Stray SM, et al. Human platelet glycoprotein V: its role in enhancing expression of the glycoprotein Ib receptor. *Blood*. 1995;86:1361-1367.
45. Kawano H, Suzuki H, Tanoue K, et al. Down-regulation and redistribution of GPV/GPv2, a subunit of von Willebrand factor receptor (GPIb/IX/V complex), on the surface membrane of thrombin-stimulated human platelets. *Br J Haematol*. 1999;104:55-63.
46. Takafuta T, Fujimura K, Kawano H, et al. Expression of platelet membrane glycoprotein V in human megakaryocytes and megakaryocytic cell lines: a study using a novel monoclonal antibody against GPV. *Thromb Haemost*. 1994;72:762-769.
47. Ramakrishnan V, Reeves PS, DeGuzman F, et al. Increased thrombin responsiveness in platelets from mice lacking glycoprotein V. *Proc Natl Acad Sci U S A*. 1999;96:13336-13341.
48. Katsutani S, Fujimoto TT, Noda M, et al. Cloning and characterization of the gene encoding the murine glycoprotein V: the conserved thrombin-cleavable protein on platelet surface. *Thromb Res*. 1998;92:43-51.
49. Ravanat C, Freund M, Mangin P, et al. GPV is a marker of in vivo platelet activation study in a rat thrombosis model. *Thromb Haemost*. 2000;83:327-333.
50. Moog S, Mangin P, Lenain N, et al. Platelet glycoprotein V binds to collagen and participates in platelet adhesion and aggregation. *Blood*. 2001;98:1038-1046. ■



REUSE THIS
CONTENT

To photocopy, e-mail, post on Internet or
distribute this or any part of *JNMA*, please
visit www.copyright.com.