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Thrombopoietin and Mean Platelet Volume in Coronary Artery Disease

HANDAN ŞENARAN, M.D., MEHMET ILERI, M.D.,* AHMET ALTINBAŞ, M.D.,† ALI KOŞAR, M.D., ERTAN YETKIN, M.D.,*
MUSTAFA ÖZTÜRK, M.D., YAŞAR KARAASLAN, M.D., ŞERAFETTIN KIRAZLI, PH.D.

Fatih University Medical School Department of Hematology; *Türkiye Yüksek Ihtisas Hospital Department of Cardiology; †Süleyman Demirel University Medical School, Department of Cardiology, Ankara, Turkey

Summary

Background: Large platelets are shown to be hemostatically more active. It has been suggested that mean platelet volume (MPV) is increased during acute myocardial infarction (AMI) and unstable angina pectoris (USAP). However, the underlying mechanism of the phenomenon remains unclear.

Hypothesis: In this study, platelets, MPV, and thrombopoietin (TP) levels were investigated in patients with coronary artery disease (CAD) and healthy controls.

Methods: Twenty patients with AMI and 20 patients with USAP were included in this study. Seventeen healthy adult subjects served as controls. Venous blood samples of the subjects were drawn within 12 h after admission. Thrombopoietin levels were measured by ELISA and platelet counts and MPV were assayed by autoanalyzer.

Results: Patients with AMI and USAP had higher platelet counts than those in the control group. Although the platelet counts were slightly higher in AMI than in USAP, this did not reach statistical significance. Mean platelet volume and levels of TP were found to be elevated in patients with AMI and USAP compared with control subjects ($p < 0.001$). Thrombopoietin levels were higher in AMI than USAP, but this was not statistically significant. There was a positive correlation between TP levels and MPV values ($p < 0.05$).

Conclusion: Increased TP levels may increase both platelet counts and platelet size, resulting in hemostatically more active platelets, which may contribute to the development and progression of CAD.

Address for reprints:

Dr. Ali Koşar
Çiftlik Cad No:57 TR-06510
Emek Ankara, Turkey

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Introduction

Megakaryocytopoiesis is a perplexing enigmatic process that depends on both early- and late-acting hematopoietic growth factors. The proliferation and maturation steps of this process are regulated by lineage nonspecific megakaryocytopoietic cytokines and thrombopoietin (TP).[1,2] Thrombopoietin is the most critical cytokine regulator of platelet production and maturation.[1,2] Platelets are involved in hemostatic repair and play an essential role in arterial thrombosis and atherosclerosis.[3] Circulating platelets are heterogeneous with respect to their size, density, and reactivity; large platelets are hemostatically more active.[4] From the experimental results it has been postulated that platelet volume and megakaryocyte ploidy are influenced by separate hormonal factors in such a manner that they may be stimulated independently or together. Platelet volume changes can occur only after an alteration in the rate of platelet destruction; increase in megakaryocyte ploidy can be associated with a change in the rate of platelet production. In acute states of platelet destruction, increase in platelet volume may be a result of change in the fragmentation pattern of megakaryocyte cytoplasm.[5] In chronic states, when platelet destruction and production are stimulated together, an increase in platelet size may be associated with an increase in megakaryocyte ploidy.[6] As there is compelling evidence that changes in platelet heterogeneity are preceded by changes in megakaryocyte ploidy and cytoplasmic volume, studies have been undertaken to associate megakaryocyte changes with diseases in which platelets are involved, especially acute coronary syndromes.[7] When bone marrow biopsies were carried out in patients with acute myocardial infarction (AMI) 2 to 3 weeks after the acute event, it was found that the mean cytoplasmic volume of megakaryocytes is significantly increased compared with control subjects with noncardiac chest pain.[7]

It has been suggested that mean platelet volume (MPV) is increased during AMI and unstable angina pectoris (USAP).[8] The correlation between platelet size and hemostatic reactivity suggests that large platelets have higher thrombotic poten-

tial; however, the underlying mechanism of the phenomenon remains unclear. Thrombopoietin is the most critical cytokine regulator of platelet production and maturation and appears to be the major regulator of in vivo platelet production.[9] In this study, platelet counts, MPV values, and TP levels were investigated in patients with AMI, USAP, and in healthy controls.

Material and Methods

Study Group

Twenty patients with AMI (7 women, 13 men; mean age ± standard deviation [SD] 62 ± 9 years) and 20 patients with USAP (6 women, 14 men; mean age SD 59 ± 11 years) were included in this study. Seventeen healthy adult subjects (6 women, 11 men; mean age ± SD 57 ± 9 years) served as the control group. The patients with primary thrombocytosis, hemorrhagic diathesis, or those on anticoagulant or antiaggregant medications were excluded. The diagnosis of AMI was made on the basis of chest pain persisting for > 30 min, ST-segment elevation of 0.2 mV in at least two contiguous leads on a standard 12-lead electrocardiogram (ECG), and elevation of serum creatine kinase-MB isoenzyme level more than twice the upper limit of normal. Unstable angina was defined as chest pain accompanied by ECG changes of ischemia requiring intravenous medical therapy to control symptoms.[10] Risk factors for coronary artery disease (age, gender, hypertension, diabetes mellitus, cigarette smoking, hyperlipidemia, family history of premature coronary artery disease [CAD]) were evaluated for each patient.

Blood Sampling and Assays

At admission of the patients, peripheral venous blood samples for measuring MPV and TP were drawn with 21 G multiple drawing blood collecting needles into 3.8% 1:9 trisodium citrate containing tubes without venous occlusion. The blood samples were centrifuged immediately at 3,000 g for 15 min and the plasma was stored in several aliquotes at −70°C until assayed. Thrombopoietin (Quantikine™, R&D Systems, Minneapolis, Minn., USA) was assayed by sandwich type ELISAs. Platelet counts and MPV were measured by autocounters (Coulter Maxem, Fullertown, Calif., USA).

Statistical Analysis

The Student's *t*-test was used for comparison of the platelets, MPV, and TP levels between patients and control subjects. The Spierman test was used for correlation of TP, MPV, and platelet counts. A p value of < 0.05 was considered to indicate statistical significance.

Results

Risk factors for CAD (age, gender, hypertension, diabetes mellitus, cigarette smoking, hyperlipidemia, family history of premature CAD) in the study population are listed in Table I. Platelets, MPV, and TP levels are shown in Table II. Patients with AMI ($275 \pm 76 \times 10^9$/l) and USAP ($288 \pm 81 \times 10^9$/l) had slightly higher platelet counts than those in the control group ($257 \pm 67 \times 10^9$/l), but this did not reach statistical significance ($p > 0.05$). Although the platelet counts were slightly higher in AMI than in USAP, this did not reach statistical significance ($p > 0.05$). Mean platelet volume was found to be elevated in patients with AMI (8.2 ± 0.8 fl) and USAP (7.7 ± 0.5 fl) compared with control subjects (6.6 ± 0.6 fl, $p < 0.001$). Patients with AMI (203 ± 73 pg/ml) and USAP (184 ± 64 pg/ml) had increased levels of TP compared with control subjects (85 ± 37 pg/ml, $p < 0.001$). Thrombopoietin levels were higher in AMI than in USAP, but this was not statistically significant ($p > 0.05$). There was a positive correlation between TP levels and MPV values ($p < 0.05$) but no correlation between platelet counts and TP levels. The distribution of TP, platelet counts, and MPV values is illustrated in Figure 1.

TABLE I Baseline characteristics of the study population

Risk factors	AMI (n = 20)	USAP (n = 20)	Control (n = 17)	p value
Age (years)	62 ± 9	59 ± 11	57 ± 9	NS
Hypertension	10	7	—	—
Diabetes mellitus	9	11	—	—
Family history	14	11	8	NS
Cigarette smoking	14	15	11	NS
Male/female	13/7	14/6	10/7	NS
Cholesterol (mg/dl)	255 (182–320)	247 (154–420)	216 (145–297)	NS
HDL-C (mg/dl)	46 (25–125)	46 (32–62)	42 (26–58)	NS
LDL-C (mg/dl)	140 (50-50)	131 (72–182)	124 (60–144)	NS

Abbreviations: NS = nonspecific, AMI = acute myocardial infarction, USAP = unstable angina pectoris, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol.

TABLE II Platelet counts, mean platelet volume (MPV) values, and thrombopoietin (TP) levels in patients and control subjects

Variable	AMI (n = 20)	USAP (n = 20)	Control (n = 17)
Platelet ($\times 10^9$/l)	275 ± 76 [a]	288 ± 81 [a]	257 ± 67
MPV (fl)	8.2 ± 0.8 [b,c]	7.7 ± 0.5 [c]	6.6 ± 0.6
TP (pg/ml)	203 ± 73 [c]	184 ± 64 [c]	85 ± 37

[a] Nonspecific.
[b] $p < 0.001$ vs. USAP.
[c] $p < 0.001$ vs. control group.
Abbreviations: MPV = mean platelet volume, TP = thrombopoietin, AMI = acute myocardial infarction, USAP = unstable angina pectoris.

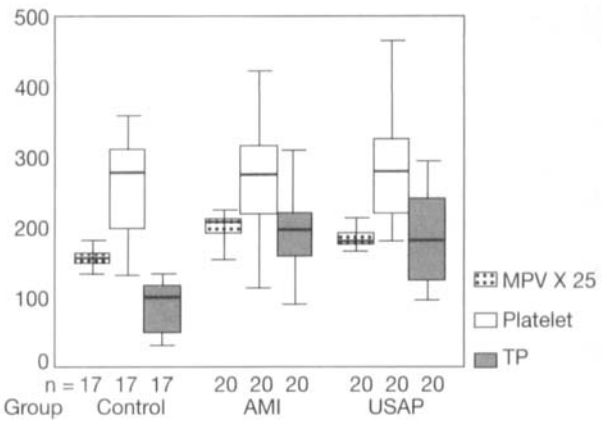

FIG. 1 The distribution of thrombopoietin, platelet, and mean platelet volume values. MPV values multiplied by 25. AMI = acute myocardial infarction, USAP = unstable angina pectoris, MPV = mean platelet volume, TP = thrombopoietin.

Discussion

The widespread availability of particle counters in clinical laboratories now permits the accurate measurement of platelet volume on a routine basis. Mean platelet volume is increased in disorders associated with accelerated platelet turnover as a result of increased numbers of megathrombocytes or in patients with Bernard-Soulier syndrome.[11,12] Some authors suggest that increased MPV provides evidence of accelerated platelet production and may be interpreted in the same manner as the reticulocyte count.[13] Platelet size is mainly determined in the production phase. Large platelets or megathrombocytes are usually noted in immune thrombocytopenic purpura and ethylene diamine tetraacetic acid (EDTA) smear or by volume determination on a Coulter counter.[14] Furthermore, MPV was found to be increased in AMI and USAP.[1,2] In a large prospective study, Martin *et al.* measured MPV in 1,716 men 6 months after AMI; values of MPV were significantly increased in those patients.[13] Mean platelet volumes measured before coronary angioplasty were shown to correlate positively with subsequent restenosis after a successful procedure.[15] In our study, MPV values were increased in patients with AMI and USAP.

As the mean life-span of thrombocytes is 10 days, elevated MPV values at the time of AMI or USAP indicate preexisting macrothrombocytosis which may contribute to the disease process; this could be due to a prior activation of megakaryocytes to release large active platelets.[16] It is suggested that increased hemostatic activity of large platelets is in part due to an increased number of glycoprotein (GP)IIb–IIIa receptors on each platelet.[17] The relationship between circulating platelets and megakaryocytes is not yet fully understood. Thrombopoetin is the major stimulant of megakaryocytopoiesis. Apart from TP, large number of cytokines including IL-3, IL-6, IL-11, erythropoietin, stemcell factor, and granulocyte-macrophage-CSF play roles in megakaryocytopoiesis.[18–21] IL-6 given to rhesus monkeys increased platelet count and size as well as megakaryocyte ploidy and size.[19] Furthermore, in addition to these numerous positive effectors, megakaryocytopoiesis might be physiologically regulated by inhibitory proteins.[20]

Thrombopoietin is produced in the liver, kidney, bone marrow, and spleen. Administration of TP results in an increase in the number of megakaryocytes in the bone marrow and spleen, in megakaryocyte size and DNA content, in megakaryocyte/platelet-specific antigen markers, and a 3- to 10-fold increase in circulating platelet concentration.[22] Numerous studies over the past 30 years have demonstrated an inverse relationship between the levels of circulating TP and platelet mass. The arrival of TP cloning, the availability of recombinant or purified protein, and the availability of gene knockout animals have allowed the dissection of this relationship at the cellular and molecular level.[23] Data from these studies best fit the model in which the predominant feedback mechanism regulating TP concentration is its binding to platelets or megakaryoctes (or both). Thus, during periods of normal homeostasis, platelet counts remain constant and circulating TP is at its basal concentration. During thrombocytopenia, platelet mass drops resulting in a reduction in the binding and degradation of TP by c-mpl positive cells[24,25] and increased concentration of free TP. Conversely, during conditions such as rebound thromboytosis or primary thrombocytemia, elevated platelet/megakaryocyte mass serves as a "sink," reducing the levels of circulating TP to achieve homeostasis.[26] Thrombopoietin production remains constant, and its concentration is regulated by the total mass of platelets/ megakaryocytes available to bind and degrade this protein.[27] Gene inactivation studies demonstrate that TP and its receptor (c-mpl) are primary regulators for megakaryothrombocytopoiesis. Thus, TP- and c-mpl-deficient mice show an approximately 85% reduction in circulating platelets and markedly reduced bone marrow megakaryocte numbers,[28] whereas studies of TP concentration in c-mpl-deficient mice show increased levels.[2] Platelets express high affinity of 200–560 pM and between 20 and 200 receptors per platelet. It has been shown that the TP level increases in plasma when the circulating platelet mass is decreased in animals and humans.[29] Moreover, upon increase of the platelet mass, the TPO level decreased rapidly; however, during thrombocytopenia TP mRNA in liver and kidney remained unchanged despite increased TP activity in the plasma.[30]

In our study, we found that MPV and TP levels were elevated in AMI and USAP. There was a statistically significant correlation between TP levels and MPV values. The authors encountered no previous studies on relationships between TP and MPV in CAD. Elevated TP levels, despite increased thrombocyte counts in our patients with CAD, were not consistent with our current knowledge. Although the underlying mechanism remains unclear, we speculate that TP levels might be influenced not only by uptake from megakaryocytes and thrombocytes but also by some undescribed factors which may cause uncontrolled TP production. Another possibility is that platelets are more damaged in patients with vascular disease. Platelet survival time is reported to be significantly shortened in patients with vascular disease, possibly due to damage of platelets while passing through the narrowed atherosclerotic arteries.[31] In such circumstances, thrombocytopoiesis may

be increased leading to formation of larger platelets and elevated levels of TP.

Conclusion

Patients with acute coronary syndromes had increased platelet counts and MPV values caused by elevated levels of thrombopoietin. The exact mechanisms and the pathophysiology need to be determined by further studies in larger series and longer follow-up of determining factors.

References

1. Kaushansky K: Thrombopoietin: The primary regulator of platelet production. *Blood* 1995;86:419–431
2. Long MW, Hoffman R: Thrombocytopoiesis. In *Haematology: Basic Principles and Practice* (Eds. Hofman R, Benz EJ, Shattil SJ, Furie B, Cohen HJ) p. 245–259. New York: Churchill Livingstone, 2000
3. Ross R: The pathogenesis atherosclerosis: A perspective for the 1990's. *Nature* 1993;62:801–809
4. Martin JF, Trowbridge EA, Salmon GL, Slater DN: The relationship between platelet and megakaryocyte volumes. *Thromb Res* 1982;287:456–459
5. Kristensen SD, Roberts KM, Kishk YT, Martin JF: Accelerated atherogenesis occurs following platelet destruction and increases in megakaryocyte size and DNA content. *Eur J Clin Invest* 1990;220: 239–247
6. Nurden P, Paujol C, Nurden AT: The evaluation of megakaryocytes to platelets. In *Baillère's Clinical Hematology. Megakaryocytes and Platelet Disorders* (Eds. Caen JP, Han ZC), p. 1–29. London: W.B. Saunders Co., 1997
7. Pizzulli L, Yang A, Martin JF, Luderitz B: Changes in platelet size and count in unstable angina compared to stable angina or non-cardiac chest pain. *Cor Art Dis* 1995;6(5):397–402
8. Halbmayer WM, Haushofer A, Radek J, Schon R, Deutsch M, Fischer M: Platelet size, fibrinogen and lipoprotein(a) in coronary heart disease. *Eur Heart J* 1998;19(1):80–84
9. Borne VDM, Folman C, Linthorst GE, Porcelijn Oudenrijn SVD, Schoot EVD: Thrombopoietin and its receptor: Structure, function and role in the regulation of platelet production. In *Baillère's Clinical Haematology. Megakaryocytes and Platelet Disorders* (Eds. Caen JP, Han ZC), p. 209–427. London: W.B. Saunders Co., 1998
10. Bruce RA: Exercise testing of patients with coronary heart disease: Principles and normal standards for evaluation. *Ann Clin Res* 1971;3:323–332
11. Threatte GA: Mean platelet volume: The need for a reference method. *Am J Clin Pathol* 1984;81:769–772
12. Trowbridge EA, Martin JF: The platelet volume distribution: A signature of the pre-thrombotic state in coronary artery disease? *Thromb Haemost* 1987;58:514–518
13. Martin JF, Bath PMW, Burr ML: Influence of platelet size on outcome after myocardial infarction. *Lancet* 1991;338:1409–1411
14. Rodgers GM, Bithell TC: The diagnostic approach to the bleeding disorders. In *Wintrobe's Clinical Haematology* (Eds. Lee GR, Foerster J, Lukens J, Paraskev F, Greer JP, Rodgers GM), p. 1157–1578. Egypt: Williams and Wilkins, 1999
15. Smyth DW, Martin JF, Michalis L, Bucknall CA, Jewitt DE: Influence of platelet size before coronary angioplasty on subsequent restenosis. *Eur J Clin Invest* 1993;23(6):361–367
16. Martin JF, Plumb J, Kilbey RS, Kiskh YT: Changes in volume and density of platelets and its relationship to volume. *Br Med J* 1983; 287:456–459
17. Giles H, Smith REA, Martin JF: Platelet glycoprotein IIb-IIIa and size are increased in acute myocardial infarction. *Eur J Clin Invest* 1994;24:69–72
18. Wendling F, Han ZC: Positive and negative regulation of megakaryocytopoiesis. In *Baillère's Clinical Haematology. Megakaryocytes and Platelet Disorders* (Eds. Caen JP, Han ZC), p. 29–45. London: W.B. Saunders Co., 1998
19. Stahl CP, Zucker FD, Evatt BL, Winton EF: Effects of human interleukin-6 on megakaryocyte development and thrombocytopoiesis in primates. *Blood* 1991;78:1467–1475
20. Caen JP, Han ZC: Megakaryocyte differentiation: Positive and negative regulation. *Cell Res* 1995;5:23–31
21. Haznedaroğlu IC, Güllü IH, Dündar SV, Kirazli Ş: The significance and distinct interactions of various growth factors in physiological and pathological megakaryocytopoiesis/ thrombocytopoiesis. *Aust NZ J Med* 1997;27:191–192
22. Eaton DL, de Sauvage FJ: Thrombopoietin: The primary regulator of megakaryocytopoiesis and thrombocytopoiesis. *Exp Hematol* 1997;25:1–7
23. de Sauvage FJ, Carver-Moore K, Luoh SM, Ryan A, Dowd M, Eaton DL, Moore MW: Physiological regulation of early and late stages of megakaryocytopoiesis by thrombopoietin. *J Exp Med* 1996;183:651–656
24. Cohen-Solal K, Villeval JL, Titeux M, Lok S, Vainchenker W, Wendling F: Constitutive expression of Mpl ligand transcripts during thrombocytopenia or thrombocytosis. *Blood* 1996;88: 2578–2584
25. Emmons RVB, Reid DM, Cohen RL, Meng G, Young NS, Dunbar CE, Shulman NR: Human thrombopoietin levels are high when thrombocytopenia is due to megakaryocyte deficiency and low when due to increased platelet destruction. *Blood* 1996;87: 4068–4071
26. Stoffel R, Wiestner A, Skoda RC: Thrombopoietin in thrombocytopenic mice: Evidence against regulation at the mRNA level and for a direct regulatory role of platelets. *Blood* 1996;87:567–573
27. Eaton DL, de Sauvage FJ: Thrombopoietin: The primary regulator of megakaryocytopoiesis and thrombocytopoiesis. *Exp Hematol* 1997;25:1–7
28. Fielder PJ, Gurney AL, Stefanich E, Marian M, Moore MW, Carver- Moore K, de Sauvage FJ: Regulation of thrombopoietin levels by c-mpl-mediated binding to platelets. *Blood* 1996;87:2154–2161
29. McCartey JM, Sprugel KH, Fox NE: Murine thrombopoietin mRNA levels are modulated by platelet count. *Blood* 1995;86: 3668–3675
30. Fielder PJ, Hass P, Nagel M: Human platelets as a model for the binding and degradation of thrombopoietin. *Blood* 1997;89:2782–2788
31. Sinzinger H, Virgolini I, Fitscha P: Platelet kinetics in patients with atherosclerosis. *Thromb Res* 1990;57:507–516