



Original article

The acute effect of the antioxidant drug “U-74389G” on mean platelet volume levels during hypoxia reoxygenation injury in rats



C. Tsompos^{a,*}, C. Panoulis^b, K. Toutouzas^c, A. Triantafyllou^d, G. Zografos^c, A. Papalois^e

^a Department of Obstetrics & Gynecology, Mesologi County Hospital, Etoloakarnania, Greece

^b Department of Obstetrics & Gynecology, Aretaieion Hospital, Athens University, Attiki, Greece

^c Department of Surgery, Ippokrateion General Hospital, Athens University, Attiki, Greece

^d Department of Biologic Chemistry, Athens University, Attiki, Greece

^e Experimental Research Centre ELPEN Pharmaceuticals, S.A. Inc., Co., Pikermi, Attiki, Greece

ARTICLE INFO

Article history:

Received 2 April 2016

Received in revised form

21 September 2016

Accepted 6 October 2016

Available online 9 November 2016

Keywords:

Hypoxia

U-74389G

Reoxygenation

Mean platelets volume

ABSTRACT

Background: This experimental study examined the effect of the antioxidant drug “U-74389G”, on a rat model and particularly in a hypoxia – reoxygenation protocol. The effects of that molecule were studied hematologically using blood mean platelets volume (MPV) levels.

Methods: 40 rats of mean weight 231.875 g were used in the study. MPV levels were measured at 60 min of reoxygenation (groups A and C) and at 120 min of reoxygenation (groups B and D). The drug U-74389G was administered only in groups C and D.

Results: U-74389G administration kept significantly increased the predicted MPV levels by $12.77 \pm 3.07\%$ ($p = 0.0001$). Reoxygenation time non-significantly decreased the predicted MPV levels by $2.55 \pm 3.71\%$ ($p = 0.4103$). However, U-74389G administration and reoxygenation time together kept significantly increased the predicted MPV levels by $7.09 \pm 1.91\%$ ($p = 0.0005$).

Conclusions: U-74389G administration whether it interacted or not with reoxygenation time kept significantly increased the predicted MPV levels. This finding has great clinical interest in blood clotting and coagulation pathophysiology.

© 2016 PBJ-Associação Porto Biomedical/Porto Biomedical Society. Published by Elsevier España, S.L.U. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Transient or permanent damage with serious implications on adjacent organs and systems may be due to tissue hypoxia – reoxygenation (HR). The use of U-74389G in HR has been a challenge for many years. However, although the progress was significant, several practical affairs have not been clarified. They include: (a) how potent U-74389G should be (b) when should it be administered and (c) at what optimal dose U-74389G should be administered. The promising effect of U-74389G in tissue protection has been noted in several HR studies. U-74389G or also known as 21-[4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl]-pregna-1,4,9(11)-triene-3,20-dione maleate salt is an antioxidant which prevents both arachidonic acid-induced and iron-dependent lipid peroxidation.¹ It protects against ischemia – reperfusion (IR) injury in organs such as animal hearts, livers

and kidneys. These membrane-associating antioxidants are particularly effective in preventing permeability changes in brain microvascular endothelial cells monolayers.² A meta-analysis of 25 published seric variables, as WBC, RBC and platelet counts, hematocrit, hemoglobin, MCH, MCHC, RbcDW, platelet-crit, PDW, glucose, creatinine, uric acid, total protein, γ GT, ALP, ACP, CPK, LDH, sodium, potassium, chloride, calcium, phosphorus and magnesium levels coming from the same experimental setting, enhanced by $0.48 \pm 17.28\%$ (p -value = 0.2377) their total catabolism after U-74389G administration for the same endpoints.^{7,8} The studied molecule U-74389G belongs to a wider family of other similar antioxidant molecules; all used in several published trials.

Mean platelet volume (MPV) is a machine-calculated measurement of the average size of platelets found in blood and is typically included in blood tests as part of the complete blood count one. Since the average platelet size is larger when the body is producing increased numbers of platelets, the MPV test results can be used to make inferences about platelet production in bone marrow or platelet destruction problems. MPV is higher when there is destruction of platelets. This may be seen in inflammatory bowel disease,³ immune thrombocytopenic purpura (ITP),

* Corresponding author at: Department of Obstetrics & Gynecology, Mesologi County Hospital, Nafpaktou Street, Mesologi 30200, Etoloakarnania, Greece.
E-mail address: Tsomposconstantinos@gmail.com (C. Tsompos).

myeloproliferative diseases and Bernard–Soulier syndrome. It may also be related to pre-eclampsia and recovery from transient hypoplasia.⁴ Abnormally low MPV values correlate with thrombocytopenia when it is due to impaired production as in aplastic anemia. In addition, low MPV, can correlate with abnormally small platelet size, sometimes a symptom of a spectrum referred to as Wiskott–Aldrich Syndrome,⁵ caused by a genetic mutation of the WAS gene. A typical range of platelet volumes is 9.7–12.8 femtolitre (fL), equivalent to spheres 2.65–2.9 μm in diameter. Normal range is given as 7.5–11.5 fL.⁶ The **aim** of this experimental study was to examine the effect of the antioxidant drug “U-74389G” on rat model and particularly in a hypoxia-reoxygenation HR protocol. The effects of that molecule were studied by measuring blood MPV levels.

Materials and methods

Animal preparation

This biomedical research was licensed by Veterinary Address of East Attiki Prefecture based on 3693/12-11-2010 & 14/10-1-2012 decisions. All substances, consumables and equipment were a grant of Experimental Research Centre of ELPEN Pharmaceuticals Co. Inc. S.A. at Pikermi, Attiki. Appropriate humanistic care was adopted for Albino female Wistar rats. Pre-experimental normal housing included ad libitum diet in laboratory for one week. Animals' pre-narcosis was preceded of continuous intra-experimental general anesthesia,^{7,8} oxygen supply, electrocardiogram and acidometry. Euthanasia did not permit post-experimental awakening and preservation of animals. Rats were randomly delivered to four experimental groups by 10 animals in each one, using following protocols of HR: Hypoxia for 45 min followed by reoxygenation for 60 min (group A); hypoxia for 45 min followed by reoxygenation for 120 min (group B); hypoxia for 45 min followed by immediate U-74389G intravenous (IV) administration and reoxygenation for 60 min (group C); hypoxia for 45 min followed by immediate U-74389G IV administration and reoxygenation for 120 min (group D). The dose of U-74389G was 10 mg/kg body mass of animals. Hypoxia was caused by laparotomic clamping inferior aorta over renal arteries with forceps for 45 min. The clamp removal re-established the inferior aorta patency and restored reoxygenation. U-74389G was administered at the time of reperfusion; through catheter of inferior vena cava. The MPV levels were determined at 60th min of reoxygenation (for A and C groups) and at 120th min of reoxygenation (for B and D groups). The sampling was performed after vena cava venipuncture by filling a 2 cc dimpled control stroke syringe with pre-set volume for withdrawal and sterile insulin needle. Then, the sample was transferred to vacuum blood collection tube (disposable vacutainer) of 2 ml containing K₂EDTA, the “anti-coagulant of choice in specimen collection and blood cell features counting” according to both NCCLS and the International Council for Standardization in Hematology. MPV levels measurements were performed by Nihon Kohden celltac a MEK-6450 K automatic hematology analyzer with pre-set rat type and cyanide-free reagents. Forty female Wistar albino rats were used (mean weight 231.875 g [Standard Deviation (SD): 36.59703 g], with minimum weight 165 g and maximum weight 320 g. Rats' weight could be potentially a confusing factor, e.g. more obese rats to have higher MPV levels. This assumption was also investigated.

Control groups

20 control rats (mean mass 252.5 g [SD: 39.31988 g]) experienced hypoxia for 45 min followed by reoxygenation.

Table 1

Weight and mean platelet volume levels and Std. Dev. of groups.

| Groups | Variable | Mean | Std. dev |
|--------|----------|---------|--------------|
| A | Weight | 243 g | 45.77724 g |
| A | MPV | 6.15 fl | 0.347211 fl |
| B | Weight | 262 g | 31.10913 g |
| B | MPV | 6.51 fl | 0.4433459 fl |
| C | Weight | 212.5 g | 17.83411 |
| C | MPV | 8.78 fl | 2.078889 fl |
| D | Weight | 210 g | 18.10463 g |
| D | MPV | 8.92 fl | 1.917638 fl |

MPV, mean platelet volume; Std. Dev, standard deviation.

Table 2

Statistical significance of mean values difference for groups (DG) after statistical standard *t* test application.

| | Variable | Difference | <i>p</i> -value |
|-----|----------|------------|-----------------|
| A–B | Weight | –19 g | 0.2423 |
| A–B | MPV | –0.36 fl | 0.0820 |
| A–C | Weight | 30.5 g | 0.0674 |
| A–C | MPV | –2.63 fl | 0.0049 |
| A–D | Weight | 33 g | 0.0574 |
| A–D | MPV | –2.77 fl | 0.0024 |
| B–C | Weight | 49.5 g | 0.0019 |
| B–C | MPV | –2.27 fl | 0.0080 |
| B–D | Weight | 52 g | 0.0004 |
| B–D | MPV | –2.41 fl | 0.0045 |
| C–D | Weight | 2.5 g | 0.7043 |
| C–D | MPV | –0.14 fl | 0.6091 |

MPV, mean platelet volume; DG, difference for groups.

Group A

Reoxygenation lasted for 60 min ($n=10$ controls rats) mean mass 243 g [SD: 45.77724g], mean MPV levels 6.15 fl [SD: 0.347211 fl] (Table 1).

Group B

Reoxygenation lasted for 120 min ($n=10$ controls rats) mean mass 262 g [SD: 31.10913 g], mean MPV levels 6.51 fl [SD: 0.4433459 fl] (Table 1).

Lazaroid (L) group

20L rats (mean mass 211.25 g [SD: 17.53755 g]) experienced hypoxia for 45 min followed by reoxygenation in the beginning of which 10 mg U-74389G/kg body weight were IV administered.

Group C

Reoxygenation lasted for 60 min ($n=10$ L rats) mean mass 212.5 g [SD: 17.83411 g], mean MPV levels 8.78 fl [SD: 2.078889 fl] (Table 1).

Group D

Reoxygenation lasted for 120 min ($n=10$ L rats) mean mass 210 g [SD: 18.10463 g], mean MPV levels 8.92 fl [SD: 1.917638 fl] (Table 1).

Statistical analysis

Every weight and MPV level group was compared with each other by statistical standard *t*-tests (Table 3). Any significant difference among MPV levels, was investigated whether owed in any potent significant weight one. The application of generalized linear models (glm) with dependant variable the MPV levels was followed. The 3 independent variables were the U-74389G or no drug administration, the reoxygenation time and both variables in combination. Inserting the rats' weight also as an independent variable at glm analysis, a significant relation resulted in ($p=0.0033$), so as

Table 3
The increasing influence of U-74389G in connection with reoxygenation time.

| Increase | 95% c. in. | Reoxygenation time | t-test | p-value glm |
|----------|------------------------------|--------------------|--------|----------------|
| 2.63 fl | 1.229718 fl to 4.030282 fl | 1 h | 0.0049 | 0.0009 |
| 2.52 fl | 1.617097 fl to 3.422903 fl | 1.5 h | 0.0000 | 0.0000 |
| 2.41 fl | 1.102374 fl to 3.717626 fl | 2 h | 0.0045 | 0.0011 |
| 0.25 fl | -0.9720322 fl to 1.472032 fl | Reoxygenation time | 0.1317 | 0.6811 |
| 1.4 fl | 0.8219751 fl to 1.978025 fl | Interaction | - | 0.0000 |

c. in., confidence interval.

Table 4
Predicted mean platelet volume levels and Std. Dev. of groups.

| Groups | Mean | Std. Dev. |
|--------|-------------|--------------|
| A | 7.329363 fl | 1.072472 fl |
| B | 6.88423 fl | 0.7288264 fl |
| C | 8.043919 fl | 0.4178185 fl |
| D | 8.102489 fl | 0.4241564 fl |

MPV, mean platelet volume; Std. Dev., standard deviation.

Table 5
Statistical significance of predicted mean platelet volume values difference for groups (DG) after statistical standard t test application.

| DG | Difference | p-value |
|-----|---------------|---------|
| A-B | 0.4451332 fl | 0.2423 |
| A-C | -0.7145558 fl | 0.0674 |
| A-D | -0.7731257 fl | 0.0574 |
| B-C | -1.159689 fl | 0.0019 |
| B-D | -1.218259 fl | 0.0004 |
| C-D | -0.05857 fl | 0.7043 |

DG, difference for groups.

to further investigation was needed. The predicted MPV values for weight were calculated and are depicted at Table 4. The differences between predicted MPV values groups calculated by standard t test are depicted at Table 5. The application of generalized linear models (glm) with dependant variable the predicted MPV levels was followed. The 3 independent variables were the same as in the first glm analysis.

Results

The first glm resulted in: U-74389G administration kept significantly increased the MPV levels by 2.52 fl [1.617097–3.422903 fl] ($p=0.0000$). This finding was in accordance with the results of standard t-test ($p=0.0000$). Reoxygenation time kept non-significantly increased the MPV levels by 0.25 fl [-0.9720322 fl to 1.472032 fl] ($p=0.6811$), also in accordance with standard t-test ($p=0.1317$). However, U-74389G administration and reoxygenation time together kept significantly increased the MPV levels by 1.4 fl [0.8219751–1.978025 fl] ($p=0.0000$). Reviewing the above and Table 2, Table 3 sums up concerning the increasing influence of U-74389G in connection with reoxygenation time. The second glm

Table 6
The predicted increasing influence of U-74389G in connection with reoxygenation time.

| Increase | 95% c. in. | Reoxygenation time | t-test | p-values glm |
|---------------|-------------------------------|--------------------|--------|-----------------|
| +0.7145558 fl | -0.0501243 fl to 1.479236 fl | 1 h | 0.0674 | 0.0653 |
| +0.9664074 fl | 0.5098173 fl to 1.422997 fl | 1.5 h | 0.0001 | 0.0002 |
| +1.218259 fl | 0.6580188 fl to 1.778499 fl | 2 h | 0.0004 | 0.0002 |
| -0.1932816 fl | -0.7457019 fl to 0.3591387 fl | Reoxygenation time | 0.3375 | 0.4831 |
| +0.5377804 fl | 0.2527449 fl to 0.8228159 fl | Interaction | - | 0.0005 |

c. in., confidence interval.

Table 7
The (%) predicted increasing influence of U-74389G in connection with reoxygenation time.

| Increase | ±SD | Reoxygenation time | p-values |
|----------|--------|--------------------|----------|
| +9.29% | ±5.07% | 1 h | 0.0663 |
| +12.77% | ±3.07% | 1.5 h | 0.0001 |
| +16.25% | ±3.81% | 2 h | 0.0003 |
| -2.55% | ±3.71% | Reoxygenation time | 0.4103 |
| +7.09% | ±1.91% | Interaction | 0.0005 |

SD, standard deviation.

resulted in: U-74389G administration kept significantly increased the predicted MPV levels by 0.9664073 fl [0.5098173–1.422997 fl] ($p=0.0001$). This finding was in accordance with the results of standard t-test ($p=0.0002$). Reoxygenation time kept non-significantly decreased the predicted MPV levels by 0.1932816 fl [-0.7457019 fl to 0.3591387 fl] ($p=0.4831$), also in accordance with standard t-test ($p=0.3375$). However, U-74389G administration and reoxygenation time together kept significantly increased the predicted MPV levels by 0.5377804 fl [0.2527449–0.8228159 fl] ($p=0.0005$). Reviewing the above and Table 5, Tables 6 and 7 sum up concerning the increasing influence of U-74389G in connection with reoxygenation time on predicted MPV levels.

Discussion

Hypoxia may influence MPV levels. Ghahremanfard et al. associated⁹ MPV levels with first acute ischemic stroke severity grading ($p=0.012$). Isik et al. correlated¹⁰ the MPV levels ranged from 6.5 fL to 11.7 fL, with slow coronary flow ($p=0.03$). Balcik et al. measured¹¹ MPV values significantly higher at 24 h after progression of ischemic stroke ($p=0.001$) than control participants. Damaske et al. indicated¹² increased MPV levels in slow coronary flow phenomenon than control ones. Ertaş et al. observed¹³ an increase in MPV levels in chronic heart failure as an independent predictor of mortality than ($p=0.004$) in control group. Açıkğöz et al. noted¹⁴ significantly higher MPV values in ischemic cardiomyopathies than control ones ($p < 0.001$). Ntaios et al. related¹⁵ the significantly increased MPV levels in acute ischemic non-lacunar stroke with poor functional outcome 24 h after. Slavka et al. indicated¹⁶ that an increased MPV value (≥ 11.01 fL) creates higher risk for ischemic heart disease. İlhan et al. found¹⁷ significant differences of MPV levels in symptomatic lacunar stroke infarct group than control one ($p < 0.01$). Mayda-Domaç et al. associated¹⁸ the MPV levels as independent risk factors with ischemic, hemorrhagic stroke and a bad prognosis ($p=0.007$). Pikija et al. associated¹⁹ higher MPV levels with larger infarct volume and greater risk of late death/dependence rates ($p=0.048$) in acute ischemic stroke. Durmaz et al. found²⁰ MPV levels higher in the aspirin non-responder stable coronary artery disease patients than responder ones. Yetkin²¹ and Muscari et al. associated²² MPV levels with ischemic electrocardiographic changes in elderly population. Bitigen et al. found²³ significantly higher isolated coronary artery ectasia (CAE) MPV values than control ones ($p < 0.0001$). Ihara et al. profoundly related^{24,25} MPV levels in patients with ischemic heart

disease but non in angiographically negative group than in positive one. Nadar et al. found²⁶ lower MPV levels in acute stroke and on follow-up, but higher mean MPV ones in uncomplicated hypertensive patients than control ones. McCabe et al. found²⁷ reticulated platelets larger than more mature 'non-reticulated' platelets in ischemic cerebrovascular disease ($p < 0.01$). Greisenegger et al. associated²⁸ the MPV levels with higher risk of severe stroke and worse outcome ($p = 0.013$). O'Malley et al. found²⁹ MPV levels higher 48 h after an acute stroke ($p < 0.001$), in available survivors and in both acute and chronic (>6 months) phase of cerebral ischemia preceded of vascular event. Kristensen et al. assessed³⁰ the increased MPV level as risk factor for acute phase of MI patients and death. Legrand et al. described³¹ the in vitro platelet fragmentation in the circulation of patients with ischemic cerebrovascular disease. Dudley et al.³² distinguished primary thrombocytopenia from reactive thrombocytosis by MPV levels. Although the fragmented discussion with the successive analysis of more than 25 studies, the constant outcome is the narrow association of high MPV values with ischemia proved across all of them. The MPV values role cannot be dissociated with platelet count, coagulation physiology and blood clotting. Indeed, U-74389G administration decreases the platelet count short-term by different significance levels, exerting a potent beneficial effect on the range of all thrombotic situations. Furthermore, Fang et al. associated³³ the increase in MPV values with increased platelet distribution, prothrombin time, activated partial thromboplastin time, thrombin time, prothrombin ratio, international normalized ratio, blood clotting time and platelet anti-aggregation, but with decreased PT%, fibrinogen level, platelet aggregation ($p < 0.05$), platelet count and platelet-crit in rats. Qureshi et al. demonstrated³⁴ a dose-dependent anticoagulation effect with significantly ($p < 0.0002$) prolonged coagulation time, bleeding time, prothrombin time, activated partial thromboplastin time, international normalized ratio and MPV values, while plasma calcium concentration and mean platelet count were significantly decreased ($p < 0.001$). Yuce et al. considered the³⁵ larger platelets hemostatically more reactive than normal size ones, increasing the propensity to thrombosis. Ouyang et al. showed³⁶ that the plasma clotting time induced by cryopreserved fresh platelet-rich plasma was significantly shorter than that induced by fresh platelet-rich plasma, evaluating the MPV variable. Teitel identified the aetiological mechanism as hypoproliferative or consumptive³⁷ for impaired fibrin clot formation and delayed bleeding after primary haemostasis characteristic of hyperfibrinolysis; after examination of the MPV morphology. van der Planken et al. found³⁸ no difference in MPV between diabetic and control platelet-rich plasma. The outer layer of platelet phospholipid membrane is more procoagulant than in the quiescent state, stimulating thrombin formation in plasma. This platelet function is called platelet procoagulant activity. Gunsilius et al. calculated³⁹ the VEGF content per 10^9 platelets at 2.51 ± 2.39 pg and as dependent on the MPV values. Van der Planken et al. associated⁴⁰ the higher MPV values of buffy coat method (BCM) concentrates ($p < 0.0005$) with higher platelet procoagulant activity than the MPV ones of platelet-rich method concentrates at any storage day. Nelson et al. measured⁴¹ significantly increased MPV values ($p < 0.01$); shortened prothrombin time by 1 s ($p < 0.01$) during the consumption period of dietary $n-3$ fatty acids salmon diet. van Wersch et al. found⁴² a significant decrease of the platelet count, MPV levels and total thrombocytic clotting capacity. Mammen et al. found⁴³ a marked post-operational increase in all clotting factors and measured near normal levels 48 h after cardiopulmonary bypass operation. Platelet counts remained low, however; the decrease in factors rarely dropped so as to compromise hemostasis (<30%). Although antithrombin levels decreased below 60%, no difficulties with heparinization were encountered. A significant drop in MPV levels was recorded only after protamine administration; restored

24 h post-operatively. The MPV levels are considered a rather insignificant variable when evaluating the clotting situations. The above literature has shown that the role of MPV variable is not subordinate than other platelet characteristics and should be taken into consideration when assessing the clotting profiles. Although the information given by all these separate studies is constant, even in combination with platelet count, coagulation physiology and blood clotting; it cannot be integrated, it is ambiguous and not clear: while the MPV augmentations increase the hemostatic reactivity and associate the ischemic situations, the coagulation is attenuated. The U-74389G administration seems to enhance this phenomenon since it increases the MPV levels. How ischemia can compromise with anticoagulation by the same drug has difficult answer. Nevertheless, the investigation of the clotting process dominant direction is very complicated studied individually only by the MPV variable. This direction cannot be predicted by any critical analysis, perhaps due to the size, limits and constrains of the present experiment. However, the effect of U-74389G at other platelet features such as the platelet counts is attenuating, causing also anticoagulation trend. The resultant effect of U-74389G on the clotting tendency of blood can be determined only by the most appropriate assays evaluating the extrinsic pathway of coagulation. These assays which unpleasantly were not included in the present experiment are the prothrombin time (PT), the prothrombin ratio (PR) and the international normalized ratio (INR) that must be evaluated in a future experiment.

Conclusion

U-74389G administration whether it interacted or not with reoxygenation time kept significantly increased the predicted MPV levels in the short term prospect of this experiment. However, the total clotting effect of U-74389G can be revealed only after the PT, PR and the INR evaluation inclusion in a future experiment.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

This study was funded by Scholarship by the Experimental Research Center ELPEN Pharmaceuticals (E.R.C.E.), Athens, Greece. The research facilities for this project were provided by the aforementioned institution.

References

- <https://www.caymanchem.com/app/template/Product.vm/catalog/75860>
- Shi F, Cavitt J, Audus KL. 21-Aminosteroid and 2-(aminomethyl)chromans inhibition of arachidonic acid-induced lipid peroxidation and permeability enhancement in bovine brain microvessel endothelial cell monolayers. *Free Radic Biol Med*. 1995;19:349–57.
- Liu S, Ren J, Han G, Wang G, Gu G, Xia Q, et al. Mean platelet volume: a controversial marker of disease activity in Crohn's disease. *Eur J Med Res*. 2012;17:27.
- Lippi G, Filippozzi L, Salvagno GL, Montagnana M, Franchini M, Guidi GC, et al. Increased mean platelet volume in patients with acute coronary syndromes. *Arch Pathol Lab Med*. 2009;133:1441–3.
- <http://primaryimmune.org/about-primary-immunodeficiencies/specificdisease-types/wiskott-aldrich-syndrome>
- <http://www.diabetesexplained.com/mean-platelet-volume.html>
- Tsompos C, Panoulis C, Toutouzas K, Triantafyllou A, Zografos G, et al. The effect of the antioxidant drug "U-74389G" on lactate dehydrogenase levels during ischemia reperfusion injury in rats. *J Anal Pharm Res*. 2016;3:00043.
- Tsompos C, Panoulis C, Toutouzas K, Zografos G, Papalois A. The trends of the antioxidant drug "U-74389G" on potassium levels during hypoxia reoxygenation injury in rats. *Period Biologorum*. 2016;118:105–10.
- Ghahremanfard F, Asghari N, Ghorbani R, Samaei A, Ghomi H, Tamadon M. The relationship between mean platelet volume and severity of acute ischemic brain stroke. *Neurosciences (Riyadh)*. 2013;18:147–51.

10. Isik T, Ayhan E, Uyarel H, Ergelen M, Tanboga IH, Kurt M, et al. Increased mean platelet volume associated with extent of slow coronary flow. *Cardiol J*. 2012;19:355–62.
11. Balcik ÖS, Bilen S, Ulusoy EK, Akdeniz D, Uysal S, İkizek M, et al. Thrombopoietin and mean platelet volume in patients with ischemic stroke. *Clin Appl Thromb Hemost*. 2013;19:92–5.
12. Damaske A, Muxel S, Fasola F, Radmacher MC, Schaefer S, Jabs A, et al. Peripheral hemorheological and vascular correlates of coronary blood flow. *Clin Hemorheol Microcirc*. 2011;49:261–9.
13. Ertaş G, Kozdağ G, Emre E, Akay Y, Ural D, Hebert K. Effect of enhanced external counterpulsation treatment on mean platelet volume in patients affected by ischemic chronic heart failure. *Blood Coagul Fibrinolysis*. 2012;23:127–31.
14. Açıköz N, Ermiş N, Yağmur J, Cansel M, Ataş H, Pekdemir H, et al. The evaluation of mean platelet volume levels in patients with idiopathic and ischemic cardiomyopathy: an observational study. *Anadolu Kardiyol Derg*. 2011;11:595–9.
15. Ntaios G, Gurer O, Faouzi M, Aubert C, Michel P. Hypertension is an independent predictor of mean platelet volume in patients with acute ischaemic stroke. *Intern Med J*. 2011;41:691–5.
16. Slavka G, Perkmann T, Haslacher H, Greisenegger S, Marsik C, Wagner OF, et al. Mean platelet volume may represent a predictive parameter for overall vascular mortality and ischemic heart disease. *Arterioscler Thromb Vasc Biol*. 2011;31:1215–8.
17. İlhan D, Ozbabalik D, Gulcan E, Ozdemir O, Gülbaçs Z. Evaluation of platelet activation, coagulation, and fibrinolytic activation in patients with symptomatic lacunar stroke. *Neurologist*. 2010;16:188–91.
18. Mayda-Domaç F, Misirli H, Yılmaz M. Prognostic role of mean platelet volume and platelet count in ischemic and hemorrhagic stroke. *J Stroke Cerebrovasc Dis*. 2010;19:66–72.
19. Pikija S, Cvetko D, Hajduk M, Trkulja V. Higher mean platelet volume determined shortly after the symptom onset in acute ischemic stroke patients is associated with a larger infarct volume on CT brain scans and with worse clinical outcome. *Clin Neurol Neurosurg*. 2009;111:568–73.
20. Durmaz T, Keles T, Ozdemir O, Bayram NA, Akcay M, Yeter E, et al. Heart rate variability in patients with stable coronary artery disease and aspirin resistance. *Int Heart J*. 2008;49:413–22.
21. Yetkin E. Mean platelet volume not so far from being a routine diagnostic and prognostic measurement. *Thromb Haemost*. 2008;100:3–4.
22. Muscari A, De Pascalis S, Cenni A, Ludovico C, Castaldini N, Antonelli S, et al. Determinants of mean platelet volume (MPV) in an elderly population: relevance of body fat, blood glucose and ischaemic electrocardiographic changes. *Thromb Haemost*. 2008;99:1079–84.
23. Bitigen A, Tanalp AC, Elonu OH, Karavelioglu Y, Ozdemir N. Mean platelet volume in patients with isolated coronary artery ectasia. *J Thromb Thrombolysis*. 2007;24:99–103.
24. Ihara A, Kawamoto T, Matsumoto K, Shouno S, Morimoto T, Noma Y. Relationship between hemostatic factors and the platelet index in patients with ischemic heart disease. *Pathophysiol Haemost Thromb*. 2006;35:388–91.
25. Ihara A, Kawamoto T, Matsumoto K, Shouno S, Hirahara C, Morimoto T, et al. Relationship between platelet indexes and coronary angiographic findings in patients with ischemic heart disease. *Pathophysiol Haemost Thromb*. 2006;35:376–9.
26. Nadar SK, Lip GY, Blann AD. Platelet morphology, soluble P selectin and platelet P-selectin in acute ischaemic stroke. The West Birmingham Stroke Project. *Thromb Haemost*. 2004;92:1342–8.
27. McCabe DJ, Harrison P, Sidhu PS, Brown MM, Machin SJ. Circulating reticulated platelets in the early and late phases after ischaemic stroke and transient ischaemic attack. *Br J Haematol*. 2004;126:861–9.
28. Greisenegger S, Endler G, Hsieh K, Tentschert S, Mannhalter C, Laluschek W. Is elevated mean platelet volume associated with a worse outcome in patients with acute ischemic cerebrovascular events? *Stroke*. 2004;35:1688–91.
29. O'Malley T, Langhorne P, Elton RA, Stewart C. Platelet size in stroke patients. *Stroke*. 1995;26:995–9.
30. Kristensen SD, Husted SE, Nielsen HK, Ravn HB, Vissinger H. Interaction between thrombocytes and blood vessel wall – significance for acute ischemic coronary syndromes. *Ugeskr Laeger*. 1995;157:2295–8.
31. Legrand C, Woimant F, Haguenu M, Caen J. Platelet surface glycoprotein changes in patients with cerebral ischemia. *Nouv Rev Fr Hematol*. 1991;33:497–9.
32. Dudley JM, Messinezy M, Eridani S, Holland LJ, Lawrie A, Nunan TO, et al. Primary thrombocythaemia: diagnostic criteria and a simple scoring system for positive diagnosis. *Br J Haematol*. 1989;71:331–5.
33. Fang J, Zhang G, Teng X, Zhang Z, Pan J, Shou Q, et al. Hematologic toxicity of Gynura segetum and effects on vascular endothelium in a rat model of hepatic veno-occlusive disease. *Zhonghua Gan Zang Bing Za Zhi*. 2015;23:59–63.
34. Qureshi IZ, Kanwal S. Novel role of puberty onset protein kisspeptin as an anti-coagulation peptide. *Blood Coagul Fibrinolysis*. 2011;22:40–9.
35. Yuç M, Cakici M, Davutoglu V, Ozer O, Sari I, Ercan S, et al. Relationship between mean platelet volume and atrial thrombus in patients with atrial fibrillation. *Blood Coagul Fibrinolysis*. 2010;21:722–5.
36. Ouyang XL, Liu JH, Pan JC, Li XJ, Ma SX, Wang HB, et al. Qualitative analysis of batch preparing cryopreserved fresh platelet rich plasma. *Zhongguo Shi Yan Xue Ye Xue Za Zhi*. 2004;12:841–4.
37. Teitel JM. Clinical approach to the patient with unexpected bleeding. *Clin Lab Haematol*. 2000;22 Suppl. 1:9–11 [discussion 30–2].
38. van der Planken MG, Vertessen FJ, Vertommen J, Engelen W, Berneman ZN, De Leeuw I. Platelet prothrombinase activity, a final pathway platelet procoagulant activity, is overexpressed in type 1 diabetes: no relationship with mean platelet volume or background retinopathy. *Clin Appl Thromb Hemost*. 2000;6:65–8.
39. Gunsilius E, Petzer A, Stockhammer G, Nussbaumer W, Schumacher P, Clausen J, et al. Thrombocytes are the major source for soluble vascular endothelial growth factor in peripheral blood. *Oncology*. 2000;58:169–74.
40. Van der Planken MG, Vertessen F, Mortelmans E, Van Bockstaele D, Muylle L, Mertens G, et al. The evolution of platelet procoagulant activity of remnant platelets in stored platelet concentrates prepared by the platelet-rich plasma method and the buffy coat method. *Ann Hematol*. 1999;78:1–7.
41. Nelson GJ, Schmidt PC, Corash L. The effect of a salmon diet on blood clotting, platelet aggregation and fatty acids in normal adult men. *Lipids*. 1991;26:87–96.
42. van Wersch JW, Kaiser V, Janssen GM. Platelet system changes associated with a training period of 18–20 months: a transverse and a longitudinal approach. *Int J Sports Med*. 1989;10 Suppl. 3:S181–5.
43. Mammen EF, Koets MH, Washington BC, Wolk LW, Brown JM, Burdick M, et al. Hemostasis changes during cardiopulmonary bypass surgery. *Semin Thromb Hemost*. 1985;11:281–92.