Journal of
HYPERTENSION RESEARCH
www.hypertens.org/jhr

Original Article
J Hypertens Res (2018) 4(4):148–155

Study of platelet aggregation in hypertensive elderly patients under antihypertensive therapy

Andreea Stela Serban*, Andreea Simona Hodorogea, Paul Gabriel Ruzsa, Cristina Petrovschi, Camelia Nicolae, Irina Parvu, Adriana Mihaela Iliesiu, Ioan Tiberiu Nanea

Cardiology Department, "Prof. Th. Burghele" Hospital, Bucharest, Romania

Received: November 10, 2018, Accepted: December 13, 2018

Abstract

In arterial hypertension, endothelial dysfunction and vascular remodeling may be associated with alteration of platelet function.

Purpose: Study of platelet aggregation parameters in hypertensive elderly patients under treatment.

Material and Method: Platelet aggregation (PA) in relation to blood pressure (BP), treatment, and echocardiographic parameteres were evaluated in elderly hypertensive patients. Parameters of PA measured by spectrophotometry at 5, 10 and 15 min after adenosine diphosphate addition were: aggregation at baseline (BAgg), maximum platelet deformation (MPD), maximal aggregation (MaxAgg), time until MaxAgg (TMaxAgg), total aggregation (TA) and disaggregation up to 15 min.

Results: 32 hypertensive patients (study group), 14 males (43.7%), mean age 73.4 ± 9.3 years, were compared to 11 healthy volunteers (control group). The PA parameters were not significantly different between groups, although BAgg and MPD were higher in hypertensive patients. Older age correlated with MPD (r = 0.35, p = 0.04) and aggregation at 5 min (r =0.3, p =0.04). BP values correlated positively with TMaxAgg (r = 0.42, p = 0.01) and negative with disaggregation (r =-0.36, p=0.04). Left ventricle EF was positively associated with MaxAgg (r =0.45, p=0.009) and MM correlated negatively with TMaxAgg (r =-0.4, p=0.01).

Conclusions: In elderly patients with high blood pressure there is an increased risk of thrombogenesis due to an early increase in platelet aggregation and by a delayed disaggregation process of circulating platelets. Further studies are necessary to see if these patients would benefit from early antiplatelet therapy, in preventing thromboembolic events.

Keywords: Platelets, thrombogenesis, aggregability, maximal deformation, adenosine diphosphate, disaggregation, high blood pressure, endothelial dysfunction

Introduction

Endothelial dysfunction and vascular remodeling are known to be central mechanisms in the patient with hypertension.[1] High blood pressure (BP) is associated with an increased incidence of atherothrombotic events, the main pathogenic mechanisms involved being thrombosis and thromboembolism. Thus, platelets play a crucial role in the pathophysiology of atherosclerosis. [2] Platelet activation occurs in the setting of hemodynamic alteration, vascular dysfunction

* Correspondence to: Dr. Andreea SERBAN, Panduri Cardiology Department, Prof. Dr. Th. Burghele Hospital, sos. Panduri nr.20, Bucharest, Romania, 050653
Tel.:0726315872, e-mail:andreea.hodorogea@gmail.com

and hyperactivity of the sympathetic nervous system. [3] A number of mediators involved in these processes are able to act on blood platelets, influencing aggregation parameters. The purpose of the study was to highlight the variation of platelet aggregation parameters in hypertensive patients under antihypertensive therapy, compared to normotensive patients.

Material and Methods

An experimental *in vivo* cellular study was conducted over a 6-month period. Thirty-two Caucasian patients with high bood pressure (HBP) from the cardiology clinics at "Prof. Th. Burghele" Hospital, Bucharest, Romania were enlisted in the study. All recruited patients met criteria for HBP according to 2018 ESC/ESH Guidelines for the management of arterial hypertension. The Ethics Committee granted approval for the study and informed consent was obtained from all subjects. Major exclusion criteria were thrombolytic therapy or anticoagulant/antiplatelet therapy in the last month. Patients with infectious, inflammatory diseases or active neoplasms diagnosed in the past 6 months have been excluded from the study. Also, the use of other drugs capable of influencing platelet function was an exclusion criterion. The control group included 11 healthy volunteers gender and age-matched and with similar cardiovascular risk factors.

Physical examination, biochemical parameters and standard echocardiographic assessement were performed on all 32 patients and 11 healthy volunteers. Body mass index (BMI), heart rate (HR), systolic (sBP) and diastolic blood pressure (dBP) were recorded before venopuncture. All patients were under optimal antihypertensive treatment,. Due to ethical reasons discontinuing the medication was not an option. Patients did not drink caffeine, alcohol and didn't smoke tobacco for at least 12 hours before venopuncture. 5 ml of peripheral venous blood was harvested at 8 am, on a 9:1 anticoagulant substrate.

Platelet separation and induction of platelet aggregation protocol.

Blood tubes were placed in a centrifuge at 26° C, 800 RPM (150g) for 15 minutes. After visual inspection, samples classified as icteric, lipemic, contaminated with hemolysed or red blood cells were excluded. 2 ml of pure platelet rich plasma (PRP) were collected in polypropylene tubes without interfering with the intermediate polymorphonuclear layer or the lower red blood cells layer.

PRP samples were left for 30 minutes to stabilize at room temperature, obtaining a steady basal trace. The remaining blood was centrifuged 15 minutes, at 2500 RPM (1500g), 26°C, obtaining 2 ml of platelet poor plasma (PPP).

Aggregation was measured as light transmission over time (transmittance) using the spectrophotometer. Platelet suspension was agitated using a magnet in a glass cuvette at 37°C, with a speed of 1000 RPM. The device was calibrated for transmission using 100% autologous PPP and 0% PRP. The agonist used - adenosine diphosphate (ADP) was added directly to the suspension to avoid creating air bubbles that may altered the transmission measurement. Initially a blank was created by dividing PPP into two 600 µL glass cuvettes, measuring transmittance at a wavelength of 633 nm. Next, one of the cuvettes was replaced with a 600 µL cuvette containing PRP. Transmission was recorded for 15 minutes (900 seconds). PA response after the addition at 60 seconds of adenosine diphosphate (ADP) 8 µL 5 mmol/L, was recorded.

An aggregation curve was obtained from which the following parameters were analysed: basal aggregation (BAgg), maximal platelet deformation (MPD), maximal aggregation (MaxAgg). In addition, we calculated the time in seconds at which the maximum aggregation occurred (TmaxAgg). Total aggregation (TA) was calculated as the distance (% transmittance) from the addition of the agonist to maximal aggregation. Disaggregation was calculated from the point of MaxAgg until second 900 (15 minutes) (Figure 1). Aggregation at 5, 10 and 15 minutes were also noted. A description of each aggregation curve was also attempted. In addition to the parameters described above, delayed platelet responses such as reversible or spontaneous aggregation have been observed.

Thrombocytosis > 600 x 109 /L may lead to artefactual inhibition of platelet aggregation [4], but since none of the samples had a platelet count > 600 x 109/L, no further dilution of PRP with PPP was required.

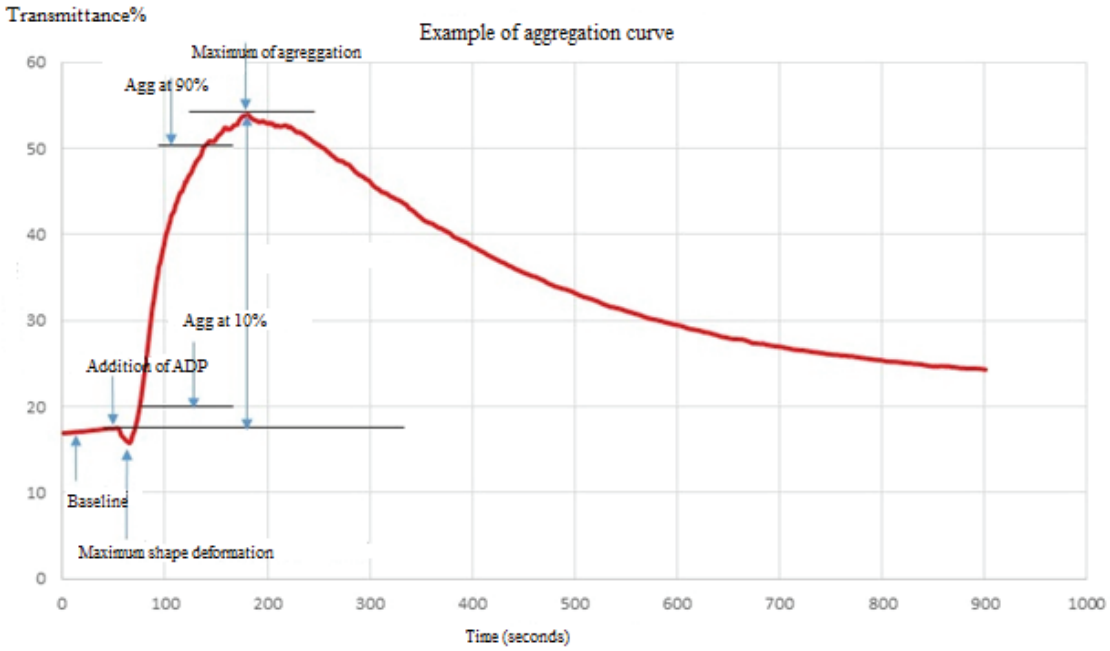

Figure 1. Example of obtained aggregation curve and analysis of parameters derived from it.
Baseline – basal state of aggregation; ADP – adenosine diphosphate, Agg at 10% - aggregation at 10% of the ascending slope of aggregation, Agg at 90% - aggregation at 90% of the ascending slope of aggregation

The results obtained were stored using the SpectraManager software. The data was transferred to a database created with the Microsoft Excel 2010 software.

Statistical analysis. Statistical analyses and graph preparation were carried out using Microsoft Excel and SPSS statistical software version 24. In normally distributed groups the results were expressed as mean ± SD. Differences between groups were assessed by Wilcoxon signed-rank test, independent-sample Mann Whitney U test and one-way analysis of variance Kruskal-Wallis H test. Pearson correlation coefficient and Spearman's rho test were used to test the strength of associations between different numerical variables. Strength of association between categorical variables was done using Chi-square and Phi and Cramer's test. The statistical significance threshold was chosen for a p-value <0.05.

Results

32 patients, 14 men (43.7%), mean age 73.4 ± 9.3 years, of which 3 patients with grade I hypertension (10%), 11 patients with grade II hypertension (34%) and 18 patients with high blood pressure III (56%) were included. 4 patients followed monotherapy, while the others were under dual therapy (12 patients) or triple therapy (16 patients). Types of drugs used are depicted in Figure 2. Characteristics of the study group and control group are described in Table 1. SBP values were significantly increased in the study group versus the control group (146.8 ± 18.4 vs. 121.8 ± 16 mmHg, p<0.001), and dBP values were significant increased in the study group (87.7 ± 12.9 vs. 73.6 ± 11.2 mmHg, p<0.001) (Figure 3).

Although baseline PA and maximal shape deformation were higher in the study group, there were no statistically significant differences of PA parameters analysed in the two groups (Table 2). The value of BP measured in mmHg and the associated type of treatment did not influence PA parameters. Platelet aggregation curves for the study group are depicted in Figure 4 and for the control group in Figure 5.

SBP had a positive correlation correlated positively with TAggMax (r = 0.42, p= 0.01) (Figure 6) and a negative correlation with disaggregation up to 15 minutes (r = -0.36, p = 0.04). The left ventricle ejection fraction (LVEF) was positively associated with AggMax

Table 1. Study group and control group characteritics

	Study group (n=32)	Control group (n=11)	P*
Mean (SD) age (years)	73.4 ± 9	76.7 ± 9	0.174
Male sex (%)	14 (44%)	5 (45%)	0.092
sBP (mmHg)	146.8 ± 18.4	121.8 ± 16	<0.001
dBP (mmHg)	87.7 ± 12.9	73.6 ± 11.2	<0.001
Smokers (%)	11 (34%)	5 (42%)	0.454
Overweight (%)	19 (61%)	7 (67%)	0.754
Diabetes (%)	11 (34%)	4 (33%)	1
Dyslipdemia (%)	24 (75%)	9 (83%)	0.18
LVEF	47.5 ± 12	53.9 ± 18.2	0.028
RWT	0.39 ± 0.11	0.45 ± 0.09	0.011
LV indexed mass (g/m^2)	117.25 ± 38.3	104.27 vs 38.6	0.39

sBP- systolic blood pressure; dBP- diastolic blood pressure; LVEF – left ventricle ejection pressure; RWT – relative wall thickness; LV indexed mass – left ventricle mass index; SD – standard deviation;
*p significant for a value <0.05

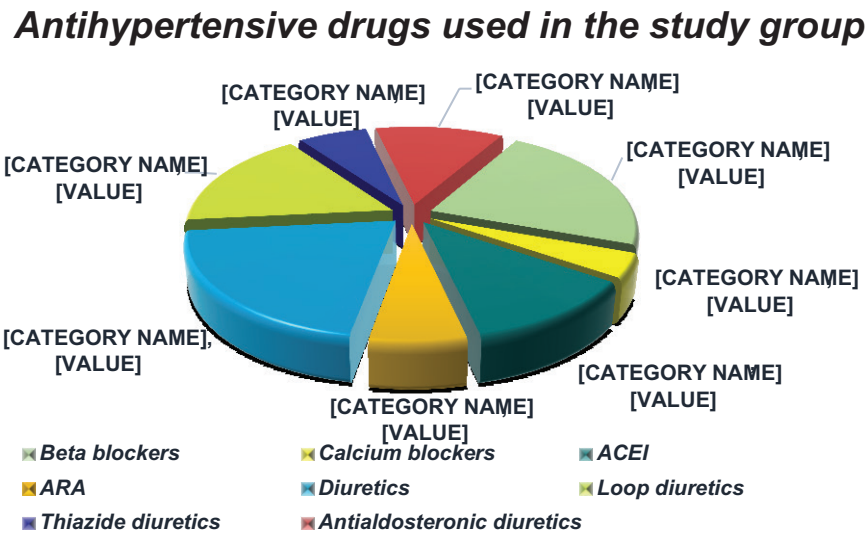

Figure 2. Antihypertensive drugs used in the study group
ACEI – angiotensin converting enzyme inhibitors
ARA – angiotensin receptor antagonists

(p = 0.009) (Figure 7) and LV mass index negative had a negative association with total aggregation (p = 0.01). Also, with older age, platelet deformation was higher (r = 0.35, p = 0.04), as did platelet aggregation at 5 minutes (r = 0.3, p = 0.04) (Figure 8).

Discussions

Although angiotensin converting enzyme inhibitors (ACEI) and angiotensin receptor blockers (ARB) are reported to have minor effects on platelet

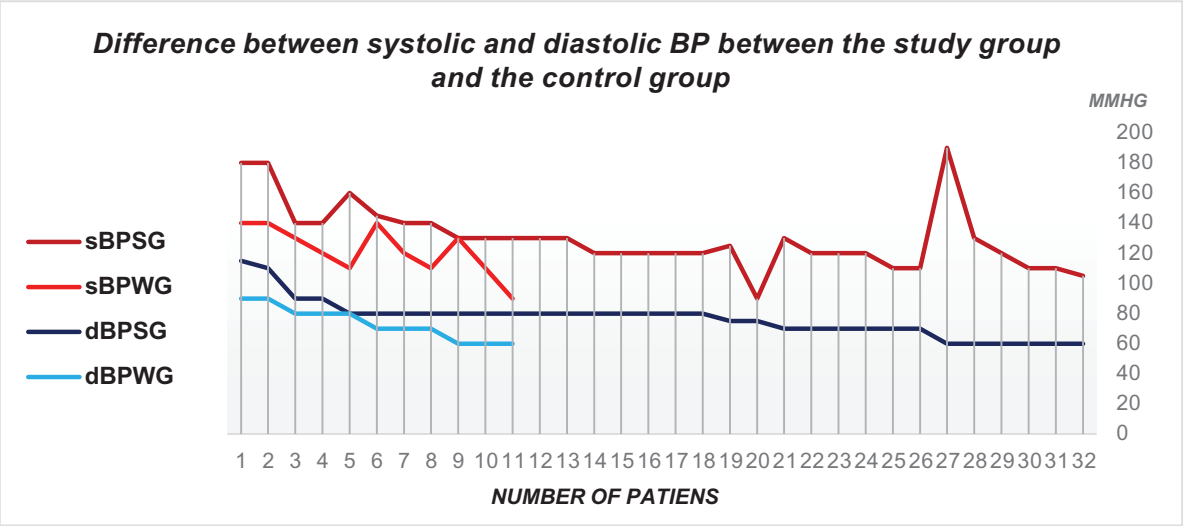

Figure 3. Difference between systolic and diastolic blood pressure between the study group and the control group
sBPSG- systolic blood pressure in the study group
sBPWG – systolic blood pressure in the witness/control group
dBPWG – diastolic blood pressure in the study group
dBPWG - diastolic blood pressure in the witness/control group

Table 2. Differences of platelet aggregation parameters between study group and control group

Parameters for platelets aggregation	Study group (n = 32)	Control group (n = ll)	p
Baseline	23.8 ± 12.6	22.4 ± 9	0.46
Maximal P1atelets deformation	22.2 ± ll.6	18.8 ± 7.8	0.48
Maximal Aggregation	60.8 ± 10.3	61-6 ± 13.3	0.97
Time untill maximal aggregation	263.9 ± 164.7	401. 2 ± 252.6	0.97
Aggregation after 5 minutes	57.4 ± 10.4	56.6 ± 14	0.8
Aggregation after 10 minutes	55.4 ± 11	56.3 ± 15.5	0.96
Aggregation after 15 minutes	52 ± 11.5	54 ± 16	0.96
Disaggregation until 15 minutes	8.7 ± 7.3	7.6 ± 8	0.88
Total Aggregation	36.9 ± ll.5	39.2. ± 16.6	0.45

function, patients who received these drugs were not excluded from the study. Studies on whole-blood aggregometry provide proof that ACEI inhibits platelet aggregation [5]. Yet, results from this study are not congruent with current literature. There was no significant correlation between the use of ACEI/ARB and platelet aggregation parameters. Also there was no difference in platelet aggregability between patients under ACEI/ARB therapy and those without, independent of the type of ACEI/ARB used. One explanation could be the small number of patients enrolled. Significant results could be obtained by further inclusion of patients.

Systolic blood pressure was associated with time until maximum aggregation, a higher systolic value

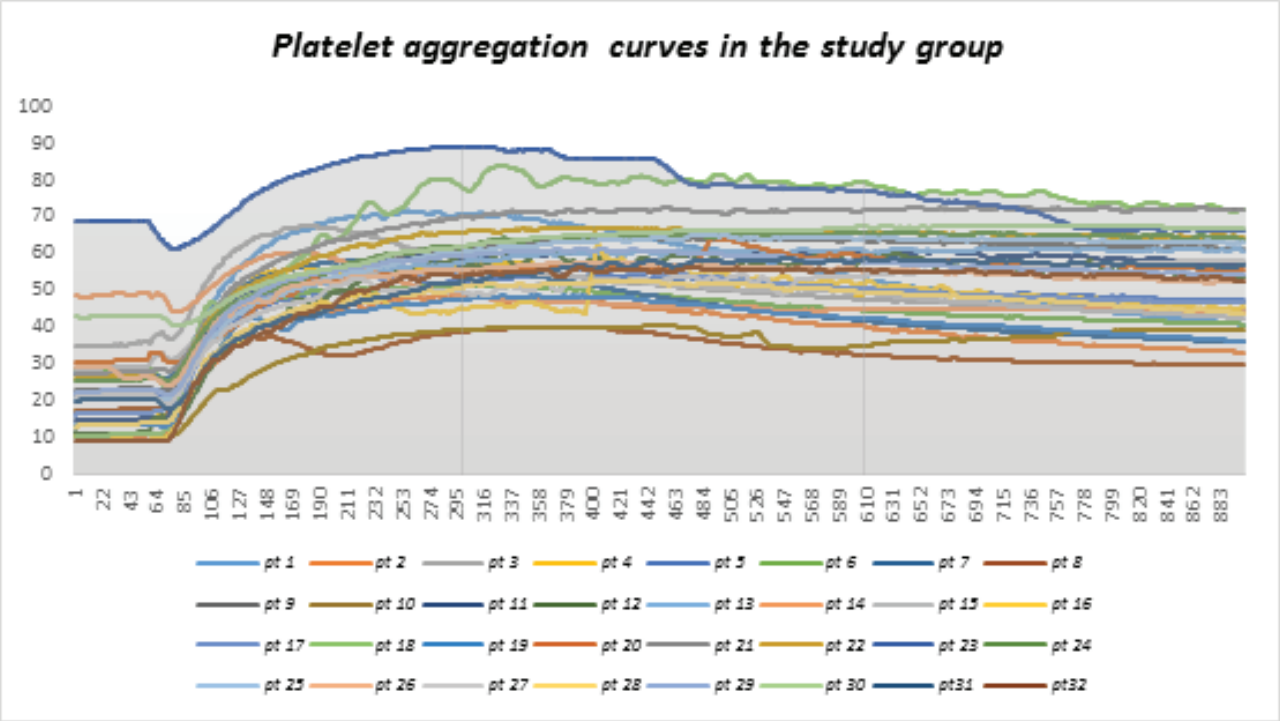

Figure 4. Platelet aggregation curves in the study group

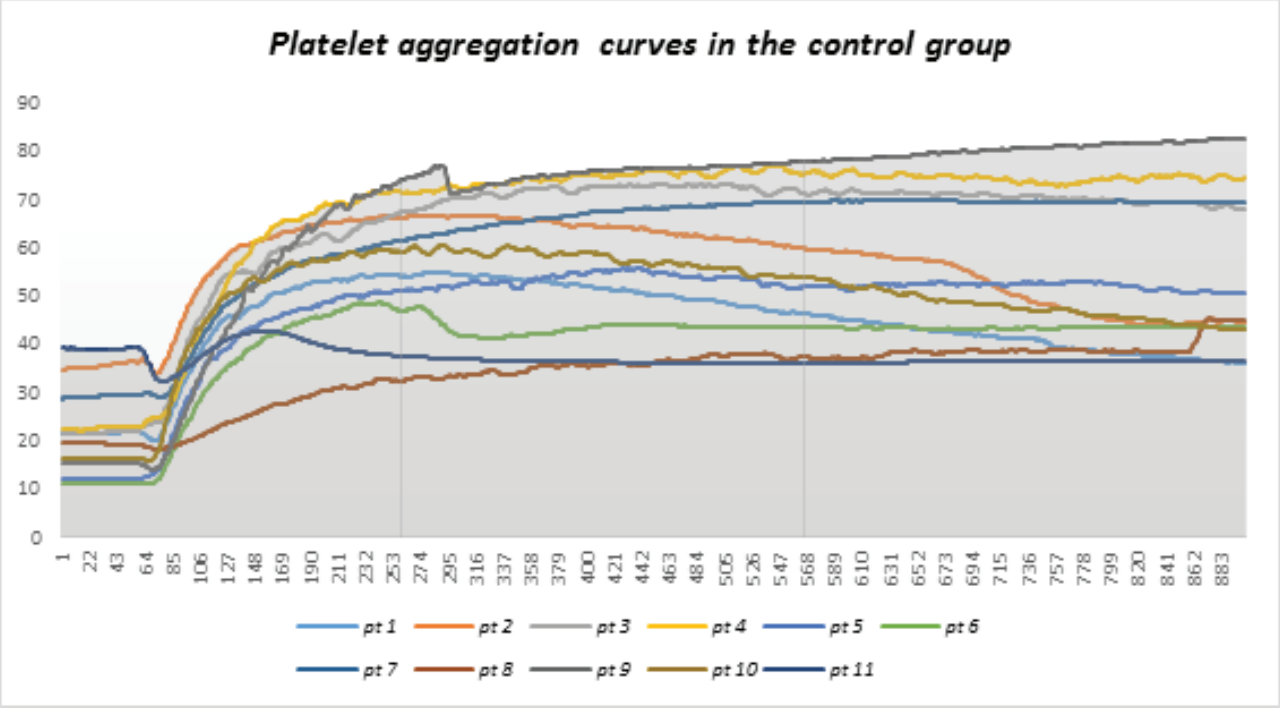

Figure 5. Platelet aggregation curves in the control group

requiring a prolonged time for platelets to reach maximal aggregation. Same was true for the disaggregation process which was slower in patients with high systolic BP values. There is no current data in literature regarding this issue.

It is well known that age and frail status are associated with changes in inflammation and coagulation, possibly altering platelet function [6]. Although other studies found no effect of chronological age on platelet aggregation amongst older

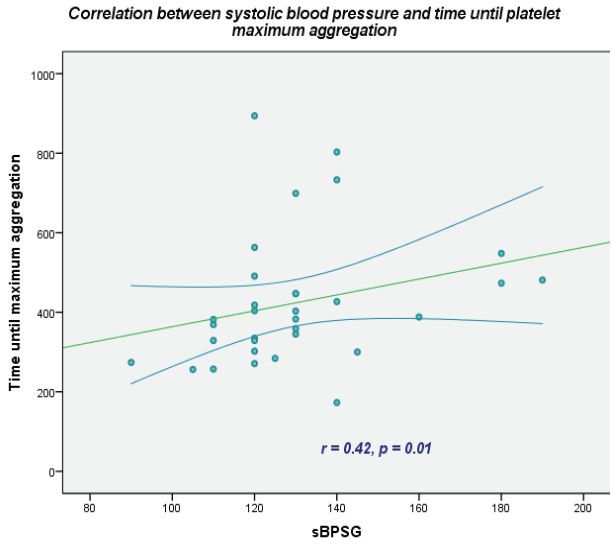

Figure 6. Correlation between systolic blood pressure and time until platelet maximum aggregation
sBPSG – systolic blood pressure in the study group

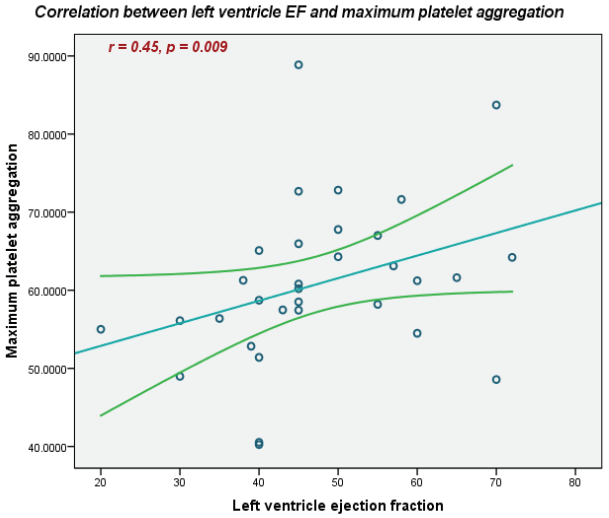

Figure 7. Correlation between left ventricle ejection fraction and time until maximum platelet aggregation
EF – ejection fraction

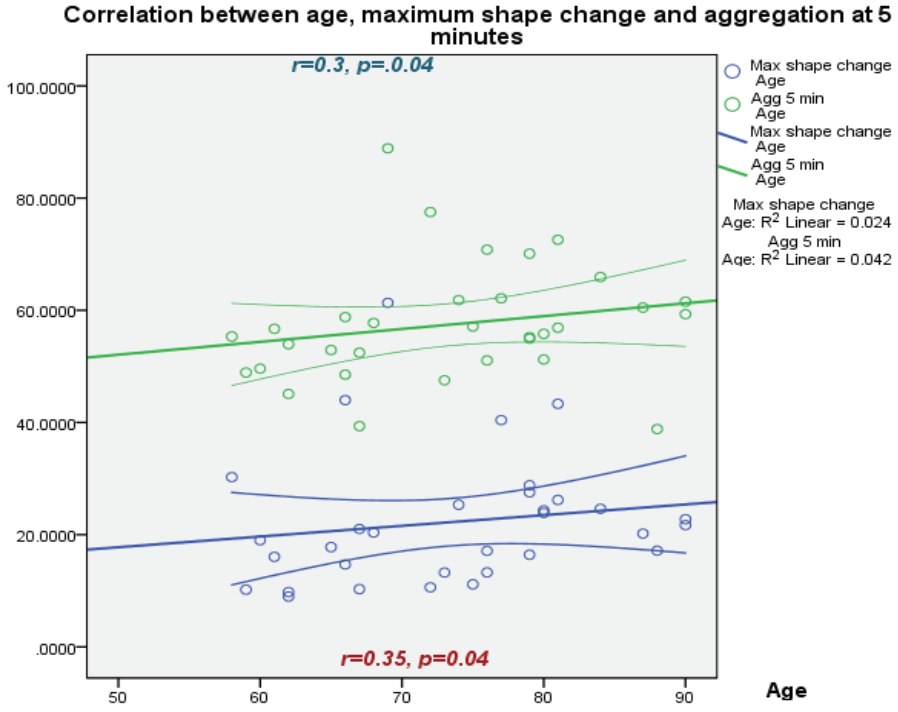

Figure 8. Correlation between age, maximum shape deformation and aggregation at 5 minutes

patients [7], in our study older age was associated with increased platelet deformation and increased aggregation in the first 5 minutes.

Conclusions

In elderly patients with high blood pressure there is an increased risk of thrombogenesis due to an

early increase in platelet aggregation and by a delayed disaggregation process of the circulating platelets. Further studies are necessary to see if these patients would benefit from early antiplatelet therapy, in preventing thromboembolic events.

Conflict of interest

The authors confirm that there are no conflicts of interest.

References

1. Widlansky, Kodlipet Dharmashankar and Michael E. 2010. "Vascular Endothelial Function and Hypertension: Insights and Directions." *Curr Hypertens Rep.* 12 (6): 448–455. doi:10.1007/s11906-010-0150-2.
2. Ferro, Eugenia Gkaliagkousi Gabriella Passacquale Stella Douma Chrysanthos ZamboulisAlbert. 2010. "Platelet Activation in Essential Hypertension: Implications for Antiplatelet Treatment." *American Journal of Hypertension* 23 (3): 229-236.
3. J.G., White. 1994. "Platelets and atherosclerosis." *Eur J Clin Invest.* 24 (1): 25-29. doi:10.1111/j.1365-2362.1994.tb02422.x.
4. Linnemann B1, Meister F, Schwonberg J, Schindewolf M, Zgouras D, Lindhoff-Last E, and MAISTHRO registry. 2008. "Hereditary and acquired thrombophilia in patients with upper extremity deep-vein thrombosis. Results from the MAISTHRO registry." *Thromb Haemost.* 100(3): 440-6. doi:10.1160/TH08-03-0196.
5. Bauriedel G1, Skowasch D, Schneider M, Andrié R, Jabs A, Lüderitz B. 2003. "Antiplatelet effects of angiotensin-converting enzyme inhibitors compared with aspirin and clopidogrel: a pilot study with whole-blood aggregometry." *Am Heart J.* 145(2): 343-348. doi:10.1067/mhj.2003.22.
6. Tu N. Nguyen, Dominic Pepperell, Marie-Christine Morel-Kopp, Robert G. Cumming, Christopher Ward,and Sarah N. Hilmer. 2016. "Effect of Frailty and Age on Platelet Aggregation and Response to Aspirin in Older Patients with Atrial Fibrillation: A Pilot Study." *Cardiol Ther.* 5(1): 51–62. doi:10.1007/s40119-016-0056-4.
7. Palomo I1, Toro C, Alarcón M. 2008. "The role of platelets in the pathophysiology of atherosclerosis (Review)." *Mol Med Rep.* 1(2): 179-184. doi:10.3892/mmr.1.2.179.