RESEARCH ARTICLE

High-on-Aspirin Residual Platelet Reactivity Evaluated Using the Multiplate® Point-of-Care Device

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Objective: The aim of this study was to evaluate the prevalence of aspirin non-responsiveness using whole blood multiple electrode aggregometry and to investigate the role of different clinical and laboratory variables associated with the lack of response. **Methods:** The present study included 116 aspirin treated patients presented with acute coronary syndromes or stroke. Response to aspirin was assessed by impedance aggregometry using arachidonic acid as agonist, in a final concentration of 0.5 mM (ASPI test). **Results:** In our data set 81% (n=94) were responders and 19% (n=22) non-responders showing high-on-aspirin platelet reactivity. Correlation analysis showed that the ward of admittance, low-density lipoproteins (LDL), concomitant antibiotic treatment, beta-adrenergic receptor blockers, history of myocardial infarction as well as PCI performed on Cardiology patients have different degrees of association with aspirin response. **Conclusion:** Concomitant treatment with beta-adrenergic receptor inhibitors, history of myocardial infarction and Cardiology ward admittance significantly increased the chance of responding to aspirin treatment whereas antibiotic therapy and low-density lipoproteins cholesterol seemed to increase the risk of high-on-aspirin residual platelet reactivity.

Keywords: aspirin, aspirin resistance, ischemic stroke, acute coronary syndrome, platelet function tests

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Introduction

Aspirin plays a central role in treatment and prevention of atherothrombotic events like stroke and acute coronary syndromes [1–3]. Randomised clinical trials have shown that aspirin reduces the risk of new atherothrombotic events by 25–30% [4]. However, 12.5% of patients develop a recurrent ischemic event during the two-year follow-up, indicating that aspirin is ineffective in certain patients [5].

Platelet aggregation measurements have shown large variability in platelet response in case of aspirin treated patients, 1–60% of patients demonstrating insufficient platelet inhibition [4]. This variation can be partially explained by differences in methods and cut-off values used to evaluate and define aspirin response, as well as by the varying extent of compliance [5].

Patients who despite treatment present a normal platelet aggregation are often referred to as aspirin non-responders, having high-on-treatment platelet reactivity or aspirin resistance [4]. With regard to aspirin resistance, the following situations have been previously reported: laboratory resistance (non-responsiveness) – evaluated using a laboratory test, chemical ("true") resistance – the inability of aspirin to acetylate platelet cyclooxygenase-1, and clinical resistance (aspirin ineffectiveness) – development of acute thrombotic events during treatment [6]. Taking into consideration that various studies demonstrated an important inhibition of thromboxane (TX) B₂ production in aspirin

treated patients detected as non-responders in platelet function tests, the term "resistance" seems inappropriate in these cases [4].

The aim of the study was to investigate the prevalence of aspirin non-responsiveness in patients treated with aspirin using whole blood multiple electrode aggregometry.

Methods

Subjects

In the present study 116 patients with acute coronary syndromes or ischemic stroke were enrolled between September 2014 and June 2015.

The study was conducted in agreement with the Declaration of Helsinki.

The study protocol was approved by the Ethics review boards of the Emergency Institute for Cardiovascular Diseases and Transplantation Tîrgu Mureş and by Emergency County Hospital Tîrgu Mureş (nr. 4123/04.08.2014 and nr. 12247/13.06.2014) and all patients gave their written informed consent.

Plain aspirin was administered following the current guidelines for stroke and acute coronary syndromes for at least five days before analysis [7]. Compliance was investigated by a face-to-face interview.

Exclusion criteria included non-compliance, use of non-steroidal anti-inflammatory drugs or GPIIb/IIIa receptor antagonists, documented absence of GPIIb/IIIa receptor, thrombocytopenia (platelet count below 100.000/μL) or thrombocytosis (platelet count over 450.000/μL), severe renal and liver dysfunction, major surgical procedure in the previous week before enrolment.

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Blood Sampling

Samples were collected into double walled Hirudin tubes (Roche Diagnostics) and analysed within 0.5 - 3 hours of blood collection.

Platelet function assessment

Platelet aggregation was measured by Multiplate® aggregometry (Roche Diagnostics) following the manufacturer's instructions. The agonist used was arachidonic acid (a COX-1 specific method [4]) in a final concentration of 0.5 mM (ASPI test). Results were plotted as an area under the curve (AUC) and consequently interpreted.

Statistical analysis

IBM® SPSS® Statistics v22.0 (Armonk, NY, USA) software was used for statistical analysis. The chosen alpha value for statistical significance was 0.05 ($\alpha=0.05$). Continuous variables were expressed as mean $\pm$ standard deviation (SD) or median and interquartile range (for non-parametric data) whereas categorical data was represented as counts and percentage. The assumption of normal distribution for the continuous variables was verified. Comparisons between groups were performed with the 2-tailed t-Test or the Mann-Whitney U test, as appropriate. Categorical variables were compared using the Chi-square (χ^2) test (or Fisher's exact test for less than 5 expected counts). To assess the association between aspirin response and different demographic, clinical and laboratory variables we performed

correlation analysis using Pearson's product-moment or Spearman's rank-order, as appropriate. *Phi* correlation was used for binary data.

All independent variables with *r* (or *phi*) value $\geq (\pm) 0.1$ that also achieved statistical significance ($p > 0.05$) were included in a univariate regression analysis.

The binary logistic regression analysis was performed with the dependent variable obtained after dichotomizing by AUC units resulted from ASPI test at the cut-off of 40 U and considering the patients as responders ($AUC < 40$ U) or non-responders ($AUC \geq 40$ U) to aspirin therapy.

Results

In our data set 81% ($n=94$) were responders and 19% ($n=22$) non-responders showing high-on-aspirin platelet reactivity. Platelet aggregation curves for both responders and non-responders to aspirin therapy are illustrated in Figure 1 and Figure 2.

Baseline characteristics of the study population in addition to the comparisons between aspirin responders and non-responders are summarized in Table I.

Correlation analysis showed that in our data set the ward where patients were admitted, low-density cholesterol (LDL-C), concomitant antibiotic treatment, beta-adrenergic receptor blockers and a history of myocardial infarction as well as PCI performed on Cardiology patients, have different degrees of association with aspirin response (Table II).

Fig. 1. Aggregation curve corresponding to an aspirin responding patient

Multiplate® platelet function analysis - V2.03.13

Fig. 2. Aggregation curve corresponding to an aspirin non-responding patient

Table I. Baseline characteristics of the study population and comparisons between aspirin responders and non-responders. SD=standard deviation; IQR=inter-quartile range; BMI=body mass index; COPD=chronic obstructive pulmonary disease; NSTEMI=non-ST-elevation myocardial infarction; STEMI=ST-elevation myocardial infarction; PCI=percutaneous coronary interventions; AUC=area under the curve; HDL=high density lipoproteins; LDL=low density lipoproteins; (* p values less than 0.05).

Variable		Overall	Response to Aspirin		p value
			YES	NO	
Ward	Cardiology, n (%)	66 (56.9)	60 (90.9)	6 (9.1)	*0.02
	Neurology, n (%)	50 (43.1)	34 (68)	16 (32)	
Male, n (%)		67 (57.7)	55 (82.1)	12 (17.9)	0.752
Age (years), mean $\pm$ SD		63.9 $\pm$ 9.7	63.4 $\pm$ 9.3	65.9 $\pm$ 11.2	0.284
Weight (kg), mean $\pm$ SD		79.6 $\pm$ 14	79.9 $\pm$ 14.2	78 $\pm$ 13.2	0.597
BMI (kg/m ²), median; IQR		27.8; 5.4	27.75; 5.07	27.8; 4.8	0.891
Ethnicity, n (%)	Romanian	89 (76.7)	69 (77.5)	20 (22.5)	0.08
	Other	27 (23.3)	25 (92.6)	2 (7.4)	
Concomitant diseases and risk factors, n (%)	Diabetes mellitus	34 (29.3)	28 (82.4)	6 (17.6)	0.816
	Arterial hypertension	99 (85.3)	80 (80.8)	19 (19.2)	0.081
	Dyslipidaemia	41 (35.3)	33 (80.5)	8 (19.5)	0.912
	COPD	5 (4.3)	3 (60)	2 (40)	0.22
	Current smoker	40 (34.5)	36 (90)	4 (10)	0.074
	Myocardial infarction	32 (27.6)	30 (93.8)	2 (6.3)	*0.031
Previous medical history, n (%)	Stroke	31 (26.7)	23 (74.2)	8 (25.8)	0.256
	Proton pump inhibitors	37 (31.9)	29 (78.4)	8 (21.6)	0.618
	Selective serotonin re-uptake inhibitors	2 (1.7)	1 (50)	1 (50)	0.345
	Antibiotics	15 (12.9)	8 (53.3)	7 (46.7)	*0.008
	Beta-blockers	80 (69)	71 (88.8)	9 (11.2)	*0.002
	Tricyclic anti-depressants	2 (1.7)	2 (100)	0 (0)	0.49
Medication at admission, n (%)	Statins	110 (94.8)	90 (81.8)	20 (18.2)	0.319
	Calcium channel blockers	35 (30.2)	30 (85.7)	5 (14.3)	0.398
	Angiotensin converting enzyme inhibitors	85 (73.3)	70 (82.4)	15 (17.6)	0.549
	Histamine-2 receptor antagonist	30 (25.9)	26 (86.7)	4 (13.3)	0.361
	Unstable angina (cardiology only)	24 (36.4)	23 (95.8)	1 (4.2)	0.404
	NSTEMI (cardiology ward only)	14 (21.2)	12 (85.7)	2 (14.3)	0.6
Clinical presentation, n (%)	STEMI (cardiology ward only)	19 (28.8)	17 (89.5)	2 (10.5)	1.000
	Stroke (neurology ward only)	49 (98)	33 (67.3)	16 (32.7)	1.000
	PCI (cardiology ward only), n (%)	61 (92.4)	57 (93.4)	4 (6.6)	0.061
	Urea [mg/dL]	38.6; 17.1	36.2; 18	43; 21.8	0.063
Laboratory findings (serum), median; IQR	Creatinine (mg/dL)	0.85; 0.32	0.84; 0.26	0.95; 0.36	0.07
	Total bilirubin (mg/dL)	0.58; 0.47	0.57; 0.49	0.6; 0.48	0.789
	Glucose (mg/dL)	107.3; 41.7	109; 45.1	106; 21.9	0.894
	Alanine aminotransferase (U/L)	20; 16.3	21; 15.1	15; 17.2	0.108
	Aspartate aminotransferase (U/L)	21; 13.5	21; 12.6	20; 17.5	0.356
	Total cholesterol (mg/dL)	159.5; 80.4	156.5; 74.8	201.8; 116.6	0.249
	HDL cholesterol (mg/dL)	35.6; 13.8	34.6; 12.3	38.6; 16.9	0.622
	LDL cholesterol (mg/dL)	88.7; 62	86; 54.7	155; 87.4	*0.006
	Triglycerides (mg/dL)	121; 70.8	123; 87.5	106.3; 83.9	0.228
	Leukocytes ($\times 10^3/\mu\text{L}$) mean $\pm$ SD	8.09 $\pm$ 2.15	8.07 $\pm$ 2.15	8.19 $\pm$ 2.27	0.839
	Haemoglobin (g/L) mean $\pm$ SD	13.7 $\pm$ 1.6	13.8 $\pm$ 1.6	13.3 $\pm$ 1.4	0.283
	Haematocrit (%)	40.7; 5.2	40.8; 5.1	39.5; 7.1	0.52
	Platelets ($\times 10^3/\mu\text{L}$)	217; 86	213; 76	236; 165	0.11

The univariate regression analysis showed that beta-blocker treatment increases the probability of a patient responding to aspirin treatment by more than 4 times (OR 4.46, $p=0.003$) while explaining a little over 10% in response variability ($R^2=0.124$). A similar statistical behaviour was observed for the history of myocardial infarction that increased the chance of being a responder by almost 4.7 times (OR 4.69, $p=0.046$) and explains 7.5% of the observed variability ($R^2=0.075$). The ward where the patients were admitted influenced significantly the probability of response, a patient from the Cardiology ward being 4.7 times more likely to respond to aspirin therapy than Neurology patients (OR 4.7, $p=0.003$), accounting for 13% of variability ($R^2=0.13$) (Figure 3). Also, among Cardiology patients, if they received interventional coronary therapy

(PCI), the chance of falling in the responder category was 9.5 times higher (OR 9.5, $p=0.032$).

As for patients receiving antibiotic treatment the chance of being non-responder was 5 times higher compared to those without such treatment (OR 0.199, $p=0.006$), which accounts for 9.5% of variability ($R^2=0.095$). Elevated low-density lipoproteins blood levels slightly increased the chance of falling in the non-responder category but to a much less extent (OR 0.98, $p=0.005$) accounting for 21.7% of observed variability ($R^2=0.217$) (Table III).

Discussion

Multiple methods have been used to evaluate the antiplatelet effect of aspirin, such as measuring the cyclooxygenase-1 (COX-1)-dependent platelet aggregation and throm-

Table II. Correlation analysis. For continuous data we reported r correlation coefficient and for binary data phi coefficient (* p values less than 0.05).

Variable	Correlation coefficient	p value
Age	-0.1	0.284
Alanine aminotransferase	0.16	0.109
Angiotensin converting enzyme inhibitors	0.056	0.549
Antibiotics	-0.272	*0.003
Arterial hypertension	-0.014	0.081
Aspartate aminotransferase	0.092	0.358
Beta blockers	0.293	*0.002
Blood glucose	-0.013	0.895
Body mass index	0.014	0.891
Calcium channel blockers	0.078	0.398
Chronic limb ischemia at presentation	0.045	0.627
Chronic obstructive pulmonary disease	-0.114	0.22
Creatinine	-0.184	0.07
Diabetes mellitus	-0.022	0.816
Dyslipidaemia	-0.01	0.912
Ethnicity	-0.162	0.08
Haematocrit	0.067	0.523
Haemoglobin	0.111	0.283
High-density lipoproteins cholesterol	-0.049	0.624
Histamine-2 receptor blockers	0.085	0.361
History of myocardial infarction	0.2	*0.031
History of stroke	-0.105	0.256
Ischemic stroke at presentation	-0.098	0.488
Leukocyte count	-0.029	0.77
Low-density lipoproteins cholesterol	0.325	*0.005
NSTEMI at presentation	-0.094	0.446
Platelet counts	-0.166	0.111
Proton pump inhibitors	-0.046	0.618
PCI	0.308	*0.012
Selective serotonin re-uptake inhibitors	-0.105	0.259
Sex	0.031	0.735
Smoker status	0.166	0.074
Statins	0.086	0.357
STEMI at presentation	-0.032	0.796
Total bilirubin	-0.020	0.869
Total blood cholesterol	-0.128	0.252
Tricyclic anti-depressants	0.064	0.49
Triglycerides	0.143	0.231
Unstable angina at presentation	0.129	0.293
Urea	-0.192	0.063
Ward	0.29	*0.02
Weight of patients	0.076	0.443

boxane levels in serum or urine. Several of these studies showed a substantial inhibition of thromboxane (TX) B2 production in aspirin-treated patients – even in those with high platelet reactivity. Thus, the term *high-on-aspirin residual platelet reactivity* might describe more appropriately this phenomenon as opposed to “aspirin resistance”. COX-1 inhibition affects only one of the several pathways involved in platelet activation, thus these platelets can still be

Fig. 3. Comparison between patients from Cardiology and Neurology wards regarding their responder status

activated by other platelet agonists like thrombin, collagen or ADP [4].

Several studies investigating the causes of high-on aspirin platelet reactivity gave different results. Concomitant administration with non-steroidal anti-inflammatory drugs, statins, selective serotonin re-uptake inhibitors, proton pump inhibitors and angiotensin receptor blockers has been correlated with an ineffective aspirin response [8-11].

Our data did not show any effect on platelet reactivity for drugs such as statins, selective serotonin re-uptake inhibitors, proton pump inhibitors or ACE inhibitors. In contrast to these findings, Feher et al. reported that patients taking ACE inhibitors were more likely to be aspirin responders, whereas those treated with statins had more chances of falling into the non-responder category [12]. The contradictory findings may be explained by the differences regarding the number of subjects included in studies.

In the univariate logistic regression analysis of the present study, use of antibiotics or beta-blockers, LDL levels, present history of myocardial infarction, PCI performed and ward were significantly different between aspirin non-responders and aspirin sensitive patients.

As for antibiotic therapy, our data suggest that it could contribute to aspirin “resistance”, and to the best knowledge of the authors, this might be the first report of such an effect.

Beta-adrenergic receptor inhibitors (beta-blockers) have the opposite effect, increasing the chance of being a re-

Table III. Univariate logistic regression analysis. (* p<0.05).

Variable	R2	Beta	OR	95% CI for OR	p value
Antibiotics	0.095	-1.613	0.199	0.063 – 0.632	*0.006
Beta blockers	0.124	1.495	4.459	1.688 – 11.778	*0.003
History of myocardial infarction	0.075	1.545	4.687	1.028 – 21.365	*0.046
Low-density lipoproteins cholesterol	0.217	-0.021	0.979	0.965 – 0.994	*0.005
Ward	0.13	1.549	4.706	1.683 – 13.159	*0.003
PCI (Cardiology patients only)	0.127	2.251	9.5	1.215 – 74.272	*0.032

sponder by more than 4 times, these being in contrast with results reported by Uzun *et al.* [13]. A similar effect was seen for the ward where patients were admitted, with Cardiology patients being significantly more prone to be responders than those in Neurology.

The results of this study regarding the association between LDL levels and aspirin response were comparable with the results of previous studies [13-16].

The clinical relevance of high on-aspirin platelet reactivity has been previously addressed, but due to the inconsistent findings current guidelines do not recommend the routine use of platelet function tests in aspirin-treated patients, although this might have a beneficial role in high-risk patients such as those with advanced stage coronary artery disease (triple vessel disease, left main severe stenosis), diffuse atherosclerotic disease, diabetes mellitus and those with chronic renal disease undergoing percutaneous coronary intervention. Also, when pharmacodynamic interactions with other drugs are suspected (as it often is the case in critical care facilities) this approach might provide a way to correctly assess the adequacy of platelet inhibition.

Study limitations

Thromboxane B₂ serum levels were not measured, therefore compliance had to be assumed based on oral interview.

Conclusions

Concomitant therapy with beta-adrenergic receptor inhibitors, history of myocardial infarction and patients admitted to the Cardiology ward had a significantly higher chance of falling into the responder category, whereas antibiotic therapy and to a lesser extent, low-density lipoprotein cholesterol blood levels, seemed to increase the risk of high-on-aspirin residual platelet reactivity.

Baseline clinical and laboratory data might provide a useful tool for identifying some of the patients that exhibit high platelet reactivity but inconsistent findings and sometimes conflicting reports call for a careful interpretation.

While we wait for definitive trials, a predictive prognostic algorithm is necessary to individualize antiplatelet therapy.

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Conflict of interests

The authors declared no potential conflicts of interest with respect to this research, authorship and/or publication of this article.

References

1. Eikelboom JW, Hirsh J, Spencer FA, *et al.* Antiplatelet drugs: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest 2012;141(2 Suppl):e89S-119S.
2. Vandvik PO, Lincoff AM, Gore JM, *et al.* Primary and secondary prevention of cardiovascular disease: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest 2012;141(2 Suppl):e637S-68S.
3. Baigent C, Blackwell L, Collins R, *et al.* Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials. Lancet (London, England). 2009;373(9678):1849-60.
4. Pettersen A a., Arnesen H, Seljeflot I. A brief review on high on-aspirin residual platelet reactivity. Vascu Pharmacol. 2015;67-69:6-9.
5. Pedersen SB, Grove EL, Nielsen HL, *et al.* Evaluation of aspirin response by Multiplate whole blood aggregometry and light transmission aggregometry. Platelets. 2009;20(6):415-20.
6. Kovács EG, Katona É, Bereczky Z, *et al.* Evaluation of laboratory methods routinely used to detect the effect of aspirin against new reference methods. Thromb Res 2014;33(5):811-6.
7. Kernan WN, Ovbiagele B, Black HR, *et al.* Guidelines for the prevention of stroke in patients with stroke and transient ischemic attack: A guideline for healthcare professionals from the American Heart Association/ American Stroke Association. Stroke. 2014.
8. Catella-Lawson F, Reilly MP, Kapoor SC, *et al.* Cyclooxygenase inhibitors and the antiplatelet effects of aspirin. N Engl J Med 2001;345(25):1809-17.
9. Fitzgerald DJ, Maree A. Aspirin and clopidogrel resistance. Hematology Am Soc Hematol Educ Program .2007;(1):114-20.
10. Malinin AI, Ong S, Makarov LM, *et al.* Platelet inhibition beyond conventional antiplatelet agents: expanding role of angiotensin receptor blockers, statins and selective serotonin reuptake inhibitors. Int J Clin Pract. 2006;60(8):993-1002.
11. Hankey GJ, Eikelboom JW. Aspirin resistance. Lancet (London, England). 2006;367(9510):606-17.
12. Feher G, Koltai K, Papp E, *et al.* Aspirin resistance: possible roles of cardiovascular risk factors, previous disease history, concomitant medications and haemorrhological variables. Drugs Aging. 2006;23(7):559-67.
13. Uzun F, Biyik I, Akturk IF, *et al.* Antiplatelet resistance and the role of associated variables in stable patients treated with stenting. Postępy w Kardiologii interwencyjnej = Adv Interv Cardiol. 2015;11(1):19-25.
14. Liu X-F, Cao J, Fan L, *et al.* Prevalence of and risk factors for aspirin resistance in elderly patients with coronary artery disease. J Geriatr Cardiol. 2013;10(1):21-7.
15. Akoglu H, Agbaht K, Piskinpasa S, *et al.* High frequency of aspirin resistance in patients with nephrotic syndrome. Nephrol Dial Transplant. 2012;27(4):1460-6.
16. Kameda S, Sakata T, Kokubo Y, *et al.* Association of platelet aggregation with lipid levels in the Japanese population: the Suita study. J Atheroscler Thromb. 2011;18(7):560-7.