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REVIEW ARTICLE

ASPIRIN RESISTANCE

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Abstract : Aspirin reduces the odds of serious atherothrombotic vascular events and death in a broad category of high risk patients by about one quarter. The term 'aspirin resistance' has been used to describe not only an absence of the expected pharmacologic effects of aspirin on platelets but also poor clinical outcomes, such as recurrent vascular events, in patients treated with aspirin. Various factors such as genetic, nonadherence, variable response to different doses, co-morbid conditions and drug interactions are responsible for aspirin resistance. Many methods, with their limitations, are available to measure the effects on platelets. Despite treatment failures, aspirin remains the single most cost-effective drug for the secondary prevention of atherothrombotic disease. To optimize its clinical effectiveness, clinicians should be aware of the potential causes of aspirin treatment failure, prescribe aspirin in appropriate doses, and encourage patients to take aspirin, stop smoking, and avoid regular use of NSAIDs.

Key words : aspirin resistance cyclooxygenase platelet turnover hypercholesterolemia diabetes mellitus

INTRODUCTION

The history of aspirin spans ages and continents from Hippocrates analgesic for women in labour to the rediscovery of the white willow bark by English country scholar Reverend Edward Stone. Bayer chemist Felix Hoffmann reinvented aspirin for his ailing father; suburban physician L.L. Craven pioneered the prophylactic antithrombotic uses of aspirin and Sir John Vane elucidated aspirin's mechanism of action as the inhibition of prostaglandin synthetase. Although the antiplatelet action of this 'wonder drug' was recognised in the 1940's it was only in 1970's and 1980's that several studies and large scale trials showed its benefit in myocardial infarction and stroke (1). With the antiplatelet trialists collaboration studies showing direct evidence

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of benefit from antiplatelet therapy among a broad range of patients having occlusive vascular disease, with reduction in the combined outcome of any serious vascular event by about one quarter, non-fatal myocardial infarction by one third, non-fatal stroke by one quarter, and vascular mortality by one sixth, aspirin became the most commonly used antiplatelet drug. Aspirin was the most widely tested drug among the trials studied (2, 3).

Magnitude of the problem

Over the last few years several studies have shown that the response to aspirin is not uniform among the patients (4, 5, 6). It ranges from desired effect of total inhibition of platelet aggregation to partial responsiveness, to a significant lack of effect on platelet aggregation. Most of these findings are based on lab methods with some studies trying to correlate them with the clinical outcome (8, 9, 10, 11). The term ‘aspirin resistance’ has been coined to describe the phenomenon although it needs to be properly defined. Various studies have estimated the incidence of aspirin resistance to be 5–75% (12). It has been suggested that such patients have a three-fold higher risk of death, heart attack or stroke. Also, 1 in 10 high risk patient suffer from the recurrence of a vascular event within the next 2 years despite regular daily aspirin therapy (13).

Factors causing or contributing to aspirin resistance

Genetic factors

(i) Variants of the cyclo-oxygenase 1 (COX-1) gene that could possibly result in an unblocked and thus aspirin-resistant COX-1 enzyme and phenotype, are a proposed mechanism (10). However, a study comprising 68 patients in whom COX-1 gene was sequenced revealed several variants but none of the mutations were located near the catalytic site. Carriers and non-carriers of one of the mutations behaved similarly when aggregation and granule content release function were studied using collagen, ADP and arachidonic acid as agonists (14). In another study variation in COX-1 activity could account for only 6–20% of the individual aggregations. Thus it was concluded that aspirin resistance expressed as unsuppressed COX-1 activity is a rare condition in an out-patient population (15).

(ii) Increased platelet turnover elevates platelet COX-2. COX-2 is 170-fold less sensitive to inhibition by aspirin. It has been speculated that platelet aspirin resistance may be caused by COX-2 in platelets by generating critical amounts of thromboxane despite aspirin treatment. In this context it is interesting that coronary artery bypass graft (CABG) patients express transiently an immunoreactive COX-2 protein with lower molecular weight. Despite the increase in platelet COX-2 after CABG, in the above study thromboxane synthesis was not prevented by the potent and selective COX-2 inhibitor celecoxib, indicating that COX-2 does not produce functionally relevant amounts of thromboxane (6).

(iii) Polymorphisms involving platelet glycoprotein la/2a, 2b/3a receptors (17).

Activation of platelets by alternative pathways

(i) In a study among 55 patients, plasma levels of thromboxane B2 as well as markers

for oxidative stress and known platelet activators 8-isoprostane and lipid peroxidation products were significantly higher in aspirin-resistant individuals. The same study found that differences in COX-1 and COX-2 expression or a novel platelet COX-1 single nucleotide polymorphism (SNP) and the P1A/A2 SNP were unrelated to aspirin-resistance (12).

(ii) Biosynthesis of thromboxane A_2 (TXA_2) via the COX-2 pathway is known to occur in macrophages and the related cells that differentiate from circulating monocytes. These cells are the principal non-platelet site of TXA_2 biosynthesis which would remain unaffected by low dose aspirin. The formation of TXA_2 from cells in the monocyte-macrophage cell line could potentially occur in circulating monocytes, pulmonary macrophages, vascular plaque, or any other site of smoking-induced inflammation (18).

(iii) Increased platelet activity due to elevated levels of von Willebrand factor, ADP, for example, following myocardial infarction (7, 19).

(iv) Increased sensitivity of platelets to collagen, epinephrine (7, 19).

Increased turnover of platelets

Increased platelet turn over has been observed following coronary artery bypass grafting. The irreversible acetylation of a specific serine moiety (position 530) of COX-1 by aspirin needs to proceed at a high rate, because the plasma half-life of aspirin is short (20 minutes). In the study by Zimmermann et al, (16) thromboxane formation by control platelets (before CABG) was largely inhibited within 15 minutes, which is within the half-life of aspirin in blood. In contrast, platelets from patients after CABG revealed a significantly delayed inhibition of cyclooxygenase by aspirin, probably not allowing for relevant platelet inhibition before conversion of aspirin to salicylate. This observation may suggest that aspirin resistance can be overcome by a prolonged administration, such as repeated doses per day (16).

Drug interaction

Ibuprofen or indomethacin given concurrently competes with aspirin for the active binding site on COX enzyme. NSAIDs, unlike aspirin, bind reversibly at the active site of the enzyme, usually depressing platelet thromboxane formation to the degree that platelet function is impaired for only a portion of the dosing interval. When co-administered with aspirin, NSAIDs competitively inhibit the access of aspirin to the acetylation site in the platelet COX-1. This interaction may be clinically relevant, because platelet aggregation may be sustained through the thromboxane pathway even if only 10 to 15 percent of the platelets remain functional. The inhibitory effects of daily low-dose aspirin on platelets are competitively inhibited by the prolonged use of multiple daily doses of ibuprofen, even when aspirin is administered before the first dose of the NSAID (20).

Dose of aspirin

Single oral doses of 5 to 100 mg of aspirin result in dose-dependent inhibition of platelet cyclooxygenase activity, with 100 mg almost

completely suppressing the biosynthesis of thromboxane A_2 in normal subjects and in patients with atherosclerotic vascular disease. Because of the permanent nature of aspirin-induced inactivation of platelet prostaglandin G/H synthase, the inhibitory effect of repeated daily doses below is cumulative. Thus, the daily administration of 30 to 50 mg of aspirin results in virtually complete suppression of platelet thromboxane biosynthesis after 7 to 10 days. With respect to the most effective dose of aspirin for patients with cerebrovascular disease, direct comparisons revealed no differences in efficacy between doses of 300 and 1200 mg daily or between doses of 30 and 283 mg daily, although a small difference may have been missed because of the limited sample size.

Evidence supports the choice of a daily dose of 75 to 100 mg for the prevention of arterial thromboembolism in all high-risk situations. This recommendation is based on the following considerations: this dose of aspirin is somewhat in excess of the lowest amount needed to suppress thromboxane-dependent platelet activation; three separate, placebo-controlled trials of daily doses of 75 mg have been completed involving more than 4000 patients with cardiovascular or cerebrovascular disease, with consistently positive results. Unbiased, indirect comparisons of different aspirin regimens do not demonstrate a larger effect of higher doses and, in fact, suggest that the opposite may be true. Because doses lower than 2 mg per kilogram of body weight per day may take a few days to exert their full antiplatelet effect, treatment should be initiated with a higher dose (such as a single adult tablet of 300 to 325 mg or two to three children's tablets of 75 to 100 mg) when immediate suppression of platelet activation is desirable, as in the initial treatment of patients with acute myocardial infarction, unstable angina, or crescendo transient cerebral ischemic attacks.

There is evidence, however, that doses of approximately 300 mg/d produce fewer GI side effects than doses of approximately 1,200 mg/d. There is also some evidence that a dose of 30 mg/d produces fewer side effects than 283 mg/d (21).

Co-morbidities

(i) Hypercholesterolemia : A significant correlation has been shown between total serum cholesterol or LDL cholesterol and the amount of thrombin generated after aspirin treatment. In subjects with high blood cholesterol levels, thrombin generation was not affected by aspirin. Blunting of aspirin response in hypercholesterolemia might be explained by (i) alterations in platelet lipid-protein matrix that render their membrane proteins less accessible for acetylation by aspirin and (ii) changes in composition and structure of plasma lipoproteins that diminish the chance of aspirin to interact with prothrombin (22).

However, in another study of aspirin resistance done in diabetic patients platelet aggregation correlated with HDL such that patients with low HDL levels were more likely to be aspirin sensitive .But aspirin resistance (AR) was not related to total cholesterol (23).

(ii) Diabetes mellitus : Although aspirin is the most commonly used antiplatelet agent

among diabetics everyday clinical practice shows that antiplatelet pharmacological approach may not always be efficient enough in people with diabetes. Diabetic platelets respond more frequently even to sub-threshold stimuli, sooner become exhausted, consumed and finally hyposensitive, thus contributing to accelerated thrombopoesis and release of fresh hyperactive platelets. A study among 203 diabetic patients (both type 1 and 2 Diabetes Mellitus) found similar prevalence of AR .Aspirin resistance was not related to age, glycohaemoglobin, total cholesterol, or a history of cardiovascular disease. Female gender was a strong independent predictor of aspirin resistance in patients with type 1 diabetes (23).

The effect of increasing doses of aspirin has been studied among 102 type 2 diabetic patients using the *platelet function analyzer* (PFA)-100. Although, a daily dose of 100 mg aspirin effectively inhibited platelet function in a majority of diabetics, a considerable proportion of patients showed a greater platelet inhibition with the use of 300 mg aspirin (24).

Other factors

(i) Like for all the other drugs compliance is one of the major factors determining the success of aspirin therapy. Older age and use of higher doses of aspirin are independent risk factors for non compliance (25).

(ii) Aspirin resistance is more common in smokers. Smoking is associated with an increased risk of myocardial infarction and sudden death. Platelet activation and thrombosis at sites of vessel stenosis and injury or plaque disruption play a crucial role in these acute coronary events. Smoking acutely increases platelet thrombus formation on arterial media wall (simulating deep arterial injury) exposed to circulating blood under rheological conditions associated with vessel stenosis. Aspirin inhibition of platelet cyclooxygenase may not be sufficient to prevent the acute increase in platelet thrombus formation after smoking. This increase in platelet thrombus formation is associated with an enhanced aggregation response to thrombin. The acute increase in platelet thrombus formation after smoking could be related to the increased epinephrine level, which can enhance platelet aggregation despite aspirin treatment (26, 27, 28).

(iii) Upright posture and maximal exercise increase platelet aggregation. Individuals who are aspirin sensitive at rest might have exercise induced platelet activation which is resistant to aspirin (29,30).

(iv) Enteric coated aspirin formulations have been considered as a factor for aspirin resistance. The lower bioavailability and poor absorption from the higher pH of small intestine may result in inadequate platelet inhibition. However there are studies to prove the contrary as well (31, 32).

Laboratory assessment of aspirin resistance

In terms of laboratory assays, aspirin has varying effects. It does not affect platelet count, prothrombin time, or activated partial thromboplastin time. The bleeding time is not sensitive or specific, and it does not necessarily reflect the risk or severity of

surgical bleeding and has no clinical utility (33). Platelet aggregometry predictably shows an absent response to the agonist arachidonic acid and may also show changes in the response to adenosine diphosphate. Flow cytometry is sometimes useful to confirm platelet activation. The clinical utility of both standard aggregometry and flow cytometry is limited, however. Both assays are technologically intensive and expensive and require highly trained laboratory personnel. In addition, the results of both assays are subject to interpretation. Further, assay results do not necessarily correlate well with clinical outcomes (34).

There is no gold-standard laboratory test for assessing platelet function, although standard platelet aggregometry is the assay against which all others are compared. The newer assays include the following :

PFA-100 (platelet function analyzer) has been approved by the US Food and Drug Administration (FDA)- to detect platelet dysfunction, von Willebrand disease, and aspirin-induced platelet inhibition (35). It is an in vitro quantitative measurement of platelet adhesion and aggregation that requires whole blood collected in 3.8% sodium citrate. As with any laboratory test, there are limitations to the *PFA-100*. Among them are that an incorrect citrate concentration, ie, 3.2%, may shorten closure time. Also, the cut-off value to determine ASA sensitivity is poorly defined (35).

VerifyNow Aspirin Assay was previously marketed as the *Ultegra Rapid Function Platelet Assay* – aspirn(36). It is FDA-approved 'to aid in the detection of platelet dysfunction due to aspirin ingestion'. It is an in vitro semiquantitative measurement of aspirin dependent aggregation that requires whole blood collected in 3.2% sodium citrate. There are primarily 2 limitations to the *VerifyNow Aspirin Assay*. The first is that, according to the manufacturer, this test cannot be used in patients with inherited platelet defects or in patients receiving many other anti-platelet drugs. The second is that the few evaluation studies to date are faulty. In some studies, aspirin resistance has been correlated with biochemical cardiovascular injury, but there has been no systematic follow-up of those patients – outcomes data (36). In those studies that did attempt to correlate test results with clinical outcomes, there were confounding variables, such as other anti-platelet drugs (37).

Platelet Works is FDA-approved to detect platelet dysfunction due to inhibition secondary to diet, ASA, and/or other drugs (38, 39). It is an in vitro quantitative measurement of platelet activation that requires whole blood collected in 3.2% sodium citrate. *Platelet Works* also has limitations that need to be addressed. There is a very short time allowed – 10 minutes – between sample collection and assay. Unacceptably high false positive rate because of interference by dietary substances such as chocolate and red wine is also a disadvantage.

Aspirin Works is FDA-approved to detect ASA-induced inhibition of Thromboxane metabolites (40). It is an in vitro quantitative measurement of aspirin induced inhibition of TXA_2 generation. The assay requires 10 mL of freshly voided urine collected in a

preservative. The urine sample may be frozen for assay later. The test, either a radio-immunoassay (RIA) or an enzyme immunoassay (ELA), measures levels of 11-dihydro-thromboxane-B_2, a relatively stable breakdown product of TXA_2. The assay therefore indirectly measures in vivo activity of TXA_2. Reduced levels are interpreted as due to ASA effect. There are 2 potential limitations with the *Aspirin Works* assay. The first is that reference ranges of RIA assays differ from those of EIA assays. Similar to tests for lipid levels, the method for a particular patient should be the same or the results may not be interpretable. The second major possible limitation is that the interpretation of results' depends on a demarcation of the quartiles. However, patients whose results comprised the second, third, and fourth quartiles appeared to be different from patients in the control population (40).

How to deal with 'Aspirin Resistance'

Although several studies have documented the presence of aspirin resistance, it has been done using different laboratory methods which do not correlate and have not been standardized. Also the patient population in the studies was heterogenous which makes generalization of the findings difficult. So at present the best option to deal with aspirin resistance would be to try to control the reversible factors such as compliance, drug interactions, advice smoking cessation, proper management of co-morbidities and use of higher doses of aspirin (325 mg) during an acute coronary event and following coronary revascularization procedures.

However, in high risk patients with documented aspirin resistance in whom the reversible factors have already been considered and corrected, the *next* suitable option would be to use clopidogrel. Clopidogrel has been shown to be as effective as aspirin in coronary and cerebrovascular diseases. The combination of both the antiplatelet drugs has been shown to be more effective than aspirin alone in these conditions. However, the combination of clopidogrel and aspirin is not significantly more effective than aspirin alone in reducing the rate of myocardial infarction, stroke, or death from cardiovascular causes among patients with stable cardiovascular disease or multiple cardiovascular risk factors (41). The efficacy of clopidogrel in patients with aspirin resistance is unclear. The study by Eikelboom et al showed that clopidogrel has enhanced antiplatelet effect in patients whose platelets are least inhibited by aspirin. The responsiveness of platelets to thromboxane was not reduced but the response to ADP was markedly diminished, more so compared to aspirin sensitive platelets (42). However a study by Lev et al (43), which was done in patients undergoing percutaneous coronary intervention, to the contrary, showed reduced efficacy of clopidogrel in aspirin resistant patients, suggesting the occurrence of dual drug resistance. In addition, resistance to clopidogrel has also been reported (44).

Thus, it appears that it would be appropriate to prescribe aspirin to all patients at high risk of cardiovascular disorder until an accurate and inexpensive lab method towards assessing 'aspirin resistance' is devised.

REFERENCES

1. Mueller RL, Scheidt S. History of drugs for thrombotic disease. Discovery, development, and directions for the future. *Circulation* 1994; 89(1): 432–449.
2. Antiplatelet trialist's collaborative overview of randomized trials of antiplatelet therapy. Prevention of death, myocardial infarction and stroke by prolonged antiplatelet therapy in various categories of patients. *BMJ* 1994; 308: 81–106.
3. Antithrombotic trialist's collaboration. Collaborative meta-analysis of randomized trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. *BMJ* 2002; 324: 71–86.
4. Hurlen M, Seljeflot I, Arnesen H. The effect of different antithrombotic regimens on platelet aggregation after myocardial infarction. *Scand Cardiovasc J* 1998; 32(4): 233–237.
5. Tarjan J, Salamon A, Jager R, Poor F, Barcozi V, Dinnyes J et al. The rate of acetylsalicylic acid non-respondents among patients hospitalized for acute coronary disease, previously undergoing secondary salicylic acid prophylaxis *Orv Hetil* 1999; 140(42): 2339–2343.
6. Andersen K, Hurlen M, Arnesen H, Seljeflot I. Aspirin non-responsiveness as measured by PFA-100 in patients with CAD. *Thromb Res* 2002; 108(1): 37–42.
7. McCabe DJ, Harrison P, Mackie U, Sidhu PS, Lawrie AS, Purdy G et al. Assessment of the antiplatelet effects of low to medium dose aspirin in the early and late phases after ischaemic stroke and TIA. *Platelets* 2005; 16(5): 269–280.
8. Grotemeyer KH, Scharafinski HW, Hussteat IW. Two-year follow-up of aspirin responder and aspirin non responder. A pilot-study including 180 post stroke patients. *Thromb Res* 1993; 71(5): 397–403.
9. Grundmann K, Jaschonck K, Kleine B, Dichgans J, Topka H. Aspirin non-responder status in patients with recurrent cerebral ischemic attacks. *J Neurol* 2003; 250(1): 63–66.
10. Eikelboom JW, Hirsh J, Weitz JI, Johnston M, Yi Q, Yusuf S. Aspirin resistant thromboxane biosynthesis and the risk of myocardial infarction, stroke, or cardiovascular death in patients at high risk for cardiovascular events. *Circulation* 2002; 105: 1650–1655.
11. Buchanan MR, Schwartz L, Bourassa M, Brister S J, Peniston CM. Results of BRAT study-a pilot study investigating the possible significance of ASA non responders on the benefits and risks of ASA on thrombosis in patients undergoing coronary bypass surgery. *Can J Cardiol* 2000; 16: 1385–1390.
12. Kranzhofer R, Ruef J. Aspirin resistance in coronary artery disease is correlated to elevated markers for oxidative stress but not to the expression of cyclooxygenase 1/2, a novel cox-1 polymorphism or the PIA(l/2) polymorphism. *Platelets* 2006, 17(3): 163–169.
13. Gum PA, Kottke-Marchant K, Welsh PA, White J, Topol EJ. A prospective, blinded determination of the natural history of aspirin resistance among stable patients with cardiovascular disease. *J Am Coll Cardiol* 2003; 41(6): 961–965.
14. Hillarp A, Palmavist B, Lethagen S, Villoutreix BO, Mattiasson I. Mutations within the cyclooxygenase-1 gene in aspirin non-responders with recurrence of stroke. *Thromb Res* 2003; 112(5-6): 275–283.
15. Ohmori T, Yatomi Y, Nonaka T, Kobayashi Y, Madoiwa S, Mimuro J et al. Aspirin resistance detected with aggregometry cannot be explained by cyclooxygenase activity: involvement of other signaling' pathway(s) in cardiovascular events of aspirin-treated patients. *J Thromb Haemost* 2006; 4(6): 1271–1278.
16. Zimmermann N, Wenk A, Kim U, Kienzle P, Weber A-A, Gams E et al. Functional and biochemical evaluation of platelet aspirin-resistance after coronary artery bypass surgery. *Circulation* 2003; 108(5): 542–547.
17. Szczeklik A, Undas A, Sanak M, Frolow M, Wegrzyn W. Relationship between bleeding time, aspirin and the P1A1/A2 polymorphism of platelet glycoprotein 3a. *Br J Haematol* 2000; 110(4): 965–967.
18. McAdam BF, Byrne D, Morrow JD, Gates JA. Contribution of cyclooxygenase 2 to elevated biosynthesis of thromboxane A2 and prostacyclin hi cigarette smokers. *Circulation* 2005; 112(7): 1024–1029.
19. Harrison P, Mackie I, Mathur A, Robinson MS, Hong Y, Erusalimsky JD et al. Platelet hyper-function in acute coronary syndromes. *Blood Coagul Fibrinolysis* 2005; 16(8): 557–562.
20. Catella-Lawson F, Reilly MP, Kapoor SC, Cucchiara AJ, De Marco S, Tourmier B et al. Cyclooxygenase inhibitors and the antiplatelet effects of aspirin. *N Engl J Med* 2001; 345(25): 1809–1817.

21. Patrono C. Aspirin as an antiplatelet drug. *N Engl J Med* 1994; 330(18): 1287–1294.

22. Szczeklik A, Musial J, Undas A, Swadzba J, Gora PF, Piwowarska W, Duplaga M. Inhibition of thrombin generation by aspirin is blunted in hypercholesterolemia. *ATVB* 1996; 16: 948–954.

23. Mehta SS, Silver RJ, Aaronson A, Abrahamson M, Goldfine AB. Comparison of aspirin resistance in type 1 and type 2 diabetes mellitus. *Am J Cardiol* 2006; 97(4): 567–570.

24. Abaci A, Yilmaz Y, Caliskan M, Bayram F, Cetin M, Una! A, Cetin S. Effect of increasing doses of aspirin on platelet function as measured by PFA-100 in patients with diabetes. *Thromb Res* 2005: 116(6): 465–470.

25. De Schryver ELLM, Van Gijn J, Kappelle LJ, Kondstaal PJ, Algra A. Non adherence to aspirin or oral anticoagulants in secondary prevention after ischaemic stroke. *J Neurol* 2005; 252: 1316–1321.

26. Dussaillant NG, Zapata MM, Fardella BP, Conte LG, Cuneo VM. Frequency and characteristics of aspirin resistance in Chilean cardiovascular patients. *Rev Med Chil* 2005; 133(4): 409–417.

27. Mirkhel A, Peyster E, Sundun J, Greene L, Michelson AD, Hasan A, Domanski M. Frequency of aspirin resistance in a community hospital. *Am J Cardiol* 2006; 98(5): 577–579.

28. Hung J, Lam JYT, Lacoste L, Letchacovski G. Cigarette smoking acutely increases platelet thrombus formation in patients with coronary artery disease taking aspirin. *Circulation* 1995; 92: 2432–2436.

29. Feng DL, Murillo J, Jadhav P, McKenna C, Gebara OC, Lipinska I et al. Upright posture and maximal exercise increase platelet aggregability and prostacyclin production in healthy male subjects. *British Journal of Sports Medicine* 1999; 33(6): 401–404.

30. Pamuken B, Oflaz H, Acar RD, Umman S, Koylan N, Umman B, Nisanci Y. The role of exercise on platelet aggregation in patients with stable coronary artery disease: exercise induces aspirin resistant platelet activation. *J Thromb Thrombolysis* 2005; 20(1): 17–22.

31. Cox D, Maree AO, Dooley M, Conroy R, Byrne MF, Fitzgerald DJ. Effect of enteric coating on antiplatelet activity of low dose aspirin in healthy volunteers *Stroke* 2006; 37(8): 2153–2158.

32. Karha J, Rajagopal V, Kottke-Marchant K, Bhatt DL. Lack of effect of enteric coating on aspirin-induced inhibition of platelet aggregation in healthy volunteers. *Am Heart J* 2006; 51(5): 976.

33. Lehman CM, Blaylock RC, Alexander DP, Rodgers GM. Discontinuation of the bleeding time test without detectable adverse clinical impact. *Clin Chemy* 2001; 47: 1204–1211.

34. De Gaetano G, Cerletti C. Aspirin resistance: a revival of platelet aggregation tests? *J Thromb Haemost* 2003; 1: 2048–2050.

35. Jilma B. Platelet function analyzer (PFA-100): a tool to quantify congenital or acquired platelet dysfunction. *J Lab Clin Med* 2001; 138(3): 152–163.

36. Accumetrics Inc. VerifyNow (aspirin assay) package insert. *San Diego, CA;* 2004.

37. Coleman J, Wang JC, Simon JI. Determination of individual response to aspirin therapy using the Accumetrics Ultegra RPFA-ASA system. *Point of Care* 2004; 3: 77–82.

38. Chen WH, Lee P, Ng W, Tse H, Lau C. Aspirin resistance is associated with a high incidence of myonecrosis after non-urgent percutaneous coronary intervention despite clopidogrel pretreatment. *J Am Coll Cardiol* 2004 ;43: 1122–1126.

39. Curtis JP, Wang Y, Portnay EL, Masoudi FA, Havranek EP, Krumholz HM. Aspirin, ibuprofen and mortality after myocardial infarction: a retrospective cohort study. *BMJ* 2003; 327: 1322–1323.

40. Eikelboom JW, Hirsh J, Weitz JI, Johnston M, Yi Q, Yusuf S. Aspirin-resistant thromboxane biosynthesis and the risk of myocardial infarction, stroke, or cardiovascular death in patients at high risk for cardiovascular events. *Circulation* 2002; 105: 1650–1655.

41. Bhat DL, Fox KA, Hacke W, Berger PB, Black HR, Boden WE et al. Clopidogrel and aspirin versus aspirin alone for the prevention of atherothrombotic events. *N Engl J Med* 2006; 354(16): 1706–1717.

42. Eikelboom JW, Hankey GJ, Thorn J, Claxton A, Yi Q, Gilmore G et al. Enhanced antiplatelet effect of clopidogrel in patients whose platelets are least inhibited by aspirin: a randomized cross over trial. *J Thromb Haemost* 2005; 3(12): 2649–2655.

43. Lev El, Patel RT, Maresh KJ, Guthikonda S, Granada J, DeLao T et al. Aspirin and clopidogrel drug response in patients undergoing percutaneous coronary intervention. The role of dual drug resistance. *J Am Coll Cardiol* 2006; 47(1): 27–33.

44. Matetzky S, Shenkman B, Guetta V, Shechter M, Bienart R, Goldenberg I. Clopidogrel resistance is associated with increased risk of recurrent atherothrombotic events in patients with acute myocardial infarction. *Circulation* 2004; 109(25): 3171–3175.