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ASPIRIN RESISTANCE: MOLECULAR MECHANISMS & TECHNIQUES

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ABSTRACT

Aspirin became a cornerstone in the treatment of coronary artery disease and widely used in the secondary prevention of vascular events. Aspirin resistance remains a poorly defined term though clinical definition is failure of the drug to prevent an atherothrombotic event despite the regular intake of appropriate doses is a relatively common problem. Various laboratory parameters assessing its efficacy, like bleeding time, platelet reactivity, thromboxane A2 (TXA2) production and measurement of platelet aggregation have confirmed the lack of its uniform effect on the platelets. Various molecular mechanism responsible for Aspirin resistance include Insufficient suppression of COX-1, over- expression of COX-2 mRNA, Erythrocyte induced platelet activation, Genetic polymorphism of enzymes like COX-1, COX-2 or thromboxane A2 synthase. Clinical factor like non compliance of patient is also responsible for Aspirin resistance. The limitations in understanding Aspirin resistance include difficulties in assessing platelet function and aspirin resistance and contributed to variable reporting of Aspirin resistance. Therefore, the definition of Aspirin resistance requires refinement to include genetic polymorphism of various enzymes responsible for Aspirin resistance. However, various techniques for platelet analysis also require modification to understand Aspirin resistance.

INTRODUCTION: ASA (Acetyl salicylic acid) - more commonly known as aspirin - is one of the oldest medications still in wide availability. Early Hippocrates used willow leaves, rich in ASA, to relieve the aches associated with multiple illnesses in ancient Greece [1]. Reverend Edmund Stone isolated salicilin, the glycoside of salicylic acid and the active ingredient of aspirin, from the bark of a willow tree in England in 1763 [2].

This 'newly' discovered compound was named for the Salix Alba, literally white willow. In the 1800s, various scientists tried for the extraction of salicylic acid from willow bark, but it was not until 1897 that Felix Hoffmann, a German chemist working for Friedrich Bayer has developed a well-tolerated compound and ASA was born [3]. Bayer marketed aspirin in 1899 and dominated the flourishing market of pain relievers [1]. Since then, aspirin has grown to become one of the most commonly identified trade names around the globe and one of the most commercially successful drugs. In the modern medical literature, the antithrombotic effects of aspirin were first reported in the Mississippi Valley Medical Journal in 1953 [4]. Since then, numerous studies have confirmed aspirin's antiplatelet effect, establishing its therapeutic efficacy and validating its widespread use [5].

Aspirin has become a cornerstone in the treatment of coronary artery disease (CAD) [1]. The beneficial role of aspirin in the secondary prevention of vascular events is now well established [6, 7].

However, it has been recently shown that its effect may not be uniform in all patients. Various laboratory parameters assessing its efficacy, like bleeding time, platelet reactivity, thromboxane A2 (TXA2) production and measurement of platelet aggregation have confirmed the lack of its uniform effect on the platelets, among patients who manifest breakthrough events with thrombotic and embolic complications despite being on therapeutic doses. It has suggested that one out of every eight high-risk individuals may experience an event in the next 2 years despite aspirin therapy [8]. Based on this fact, the concept of aspirin resistance has emerged. Few studies have estimated that 5% to 45% of patients with vascular disease are aspirin-resistant [9-12]. This variability is due to non-standardization of methods and definition of aspirin resistance used in these studies [13].

Rationale for Antiplatelet Therapy: Platelet activation and high platelet reactivity to agonists in acute coronary syndromes and following percutaneous coronary intervention (PCI) have associated with stent thrombosis, restenosis, inflammation, and myocardial infarction [14, 15]. Platelet activation is also associated with diabetes, hypertension, and hyperlipidemia [14] . Through crosstalk with the coagulation system, platelets play a fundamental role in generating a hypercoagulable state, which has demonstrated as an independent risk factor for ischemic events following stenting [16].

Thus, on the one hand, platelets are critical for the development of clot to arrest bleeding during normal hemostasis, whereas on the other hand, uncontrolled activation and high reactivity may result in various severe ischemic events that make the platelets as a "nidus of evil." So, the rationale for antiplatelet therapy with aspirin is to prevent the development of thrombus by attenuating platelet activation and aggregation, to arrest the procoagulant activity, to reduce inflammation, and finally to promote the dissolution of thrombus [17, 18].

Mechanism of action of Aspirin: The characterized mechanism of action of aspirin occurs through permanent inactivation of the cyclooxygenase (COX) activity of prostaglandin H (PGH) synthase 1 and synthase 2, also referred to as COX-1 and COX-2, respectively [19]. These isozymes catalyze the first committed step in prostanoid biosynthesis — the conversion of arachidonic acid to PGH2. PGH2 is an unstable biosynthetic intermediate and a substrate for several downstream isomerases that generate at least five different bioactive prostanoids, including thromboxane A2 (TXA2) and prostacyclin (PGI2). By diffusing through cell membranes, aspirin enters the COX channel, a narrow hydrophobic channel connecting the cell membrane to the catalytic pocket of the enzyme.

Aspirin first binds to an arginine- 120 residue, a common docking site for all nonsteroidal anti-inflammatory drugs; it then acetylates a serine residue (serine 529 in human COX-1 and serine 516 in human COX-2) located in the narrowest section of the channel, thereby preventing arachidonic acid from gaining access to the COX catalytic site of the enzyme [20]. Higher levels of aspirin are needed to inhibit COX-2 than to inhibit COX-1 [21].

These differences may account for the need to use considerably higher doses of aspirin to achieve analgesic and anti-inflammatory effects, whereas antiplatelet effects can be obtained with daily doses as low as 30 mg [22, 23].

Aspirin Resistance: Unfortunately, "Aspirin Resistance" remains a poorly defined term. There are conflicting reports on the incidence and clinical relevance of this phenomenon, because the term is being used to refer numbers of phenomena. These include the inability of aspirin to (1) Protect patients from clinical arterial thrombotic complications, (2) Cause a prolongation of bleeding time, (3) Inhibit platelet TX biosynthesis, or (4) Produce an anticipated effect on ≥1 in vitro tests of platelet function, mainly platelet aggregation [24, 25].

Clinical definition of aspirin resistance is "failure of the drug to prevent an atherothrombotic event despite the regular intake of appropriate doses is a relatively common problem" [25, 26]. Laboratory definitions of aspirin resistance have involved detecting either the failure of aspirin's pharmacologic effect or the failure of aspirin to prevent the inhibition of platelet aggregation. Aspirin resistance, defined by its pharmacologic action, is the persistent production of

TXA2 despite therapy, as measured by the presence of TXA2 metabolites in serum or urine. In contrast, persistent platelet aggregation despite aspirin treatment defines the failure of aspirin-mediated platelet inhibition, and this may occur via non- TX-mediated pathways of platelet activation. It has been suggested that "aspirin resistance" is a misleading term, because in some situations, aspirin successfully inhibits TX synthesis, even though platelet aggregation persists [27]. The term "aspirin nonresponse" encompasses the failure of aspirin to inhibit TX synthesis and reduce platelet aggregation [28].

Types of Aspirin Resistance: Types of Aspirin resistance is provided in **table 1** given below;

TABLE: 1 TYPES OF ASPIRIN RESISTANCE

Type I Resistance [29]	Pharmacokinetic	Platelet aggregability successfully inhibited by in vivo addition of aspirin. This may be due to patient non-compliance or a range of dose–response effect between patients
Type II Resistance [29]	Pharmacodynamic	Platelet aggregability continued when in vitro aspirin was added, with the persistent formation of TXA2. This suggests that platelet activation persists despite inhibition of COX1, possibly due to COX2 production of PGH2, which can be converted to TXA2. An alternative explanation is defective COX1 binding of aspirin due to polymorphisms in the gene encoding Ser529 or Arg120.
Type III Resistance [30, 31]	Pseudoresistance	Platelet aggregability was continued even when in vitro aspirin was added, but there was successful inhibition of TXA2 formation. The likely mechanisms are non-TX mediated pathways of thrombosis and an increased sensitivity to collagen.

Various mechanisms responsible for aspirin resistance:

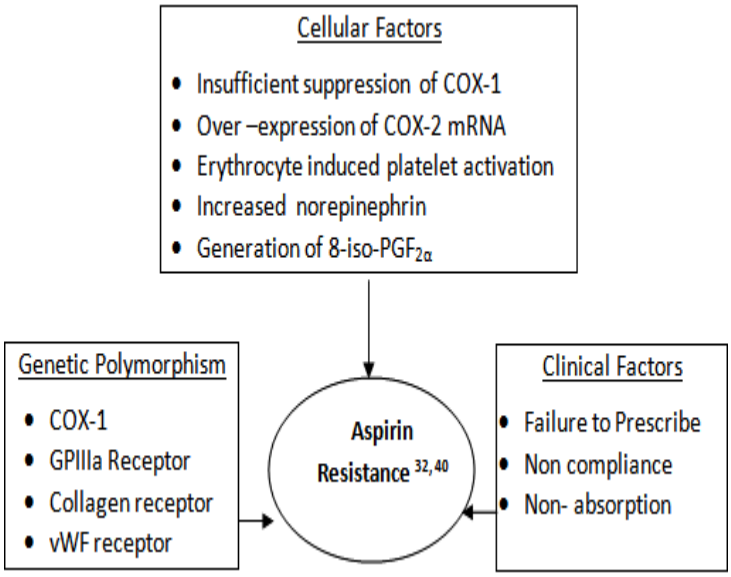

FIG. 1: VARIOUS MECHANISMS OF ASPIRIN RESISTANCE

(COX- cyclooxygenase; GP- glycoprotein; mRNA- messenger ribonucleic acid; vWF -von Willebrand factor)

Concomitant intake of other NSAIDs: Drug interaction between NSAIDS and aspirin may be one of the causes for reduced availability of aspirin at receptor site (docking site on platelet COX-1). Like aspirin, these drugs also inhibit COX-1, but unlike aspirin they are reversible inhibitors of COX. Hence their antithrombotic effect last only for a short time. Since both aspirin and NSAIDs share a common docking site on COX-1, NSAIDs when administered along with aspirin may compete with it for the active site and may reduce its antithrombotic effect. However, selective COX-2 inhibitors do not pose such problem [33].

Genetic Polymorphism: Genetic polymorphism of enzymes like COX-1, COX-2 or thromboxane A2 synthase which make them less sensitive to aspirin may be another possible cause of resistance. Aspirin resistance has been associated with the polymorphism of PLA2 gene that codes for platelet glycoprotein IIIa- a component of platelet glycoprotein IIb/IIIa [34]. Patients with this sort of resistance may derive benefit from higher dose of aspirin or from antiplatelet drugs other than aspirin like clopidogrel [35].

Reactivity of Platelets: Increased Reactivity of platelets towards other aggregating factors. Platelets can be activated by pathways other than thromboxane which are not inhibited by aspirin. Over activity of these alternate pathways of platelet activation such as erythrocyte induced platelet activation, increased sensitivity to collagen, adenosine diphosphate ,increased levels of von Willebrand factor, increased levels of noradrenalin and hyperlipidemia may be responsible for break through cardiovascular events despite aspirin therapy. Concomitant use of another antiplatelet drugs and life style modifications may be useful in these patients [36].

Rate of entry of new Platelets: Increased rate of entry of new platelets into the circulation increased risk of aspirin resistance. Clinical conditions such as myocardial infarction and coronary artery bypass surgery are associated with increased rate of entry of new platelets into the circulation introducing newly formed non aspirinated platelets into blood stream. These new platelets with an increased fraction of active COX may be responsible for aspirin resistance. Higher doses of aspirin may be more effective in such patients [37].

Alternate pathways of thromboxane synthesis: Isoenzyme G/H synthase-2(COX-2) is usually not expressed in platelets but it can be expressed in the platelets after their activation by growth factors and mediators of inflammation especially in atheromatous plaque. Low dose aspirin significantly blocks platelets COX-1 but inhibition of COX-2 require higher doses(>500mg/d). Thromboxane A2 synthesized in platelets by COX-2 may be another possible cause of aspirin resistance. Platelets can also acquire thromboxane precursors such as prostaglandin H2 from monocytes and endothelial cells and synthesize thromboxane A2, another possible cause of aspirin resistance [38].

Poor Patient Compliance: To conclude aspirin is an effective and widely used anti-platelet aggregating drug for primary and secondary prevention of atherothrombotic events. However in recent years both clinical and ex-vivo evidence has accumulated showing failure of low dose aspirin to produce expected biological response. Since aspirin resistance could have important clinical bearing, it is required to develop reliable laboratory tests to identify patients at risk for aspirin resistance and to individualize the anti-platelet therapy. At present, there are few studies to document incidence and clinical relevance of this phenomenon; more investigations are required to understand the mechanism, prevalence and clinical importance of this phenomenon [39].

Techniques for determining Aspirin Resistance:

Platelet Assays: Numerous assays have been developed to measure platelet reactivity and, hence, indirectly quantify aspirin's antiplatelet effect. However, when attempting to interpret studies investigating laboratory-identified ASA resistance, it should be taken into account that most of these assays measure platelet aggregation that may not necessarily be specific for TXA2-induced platelet reactivity. Therefore, laboratory studies may indicate an individual is ASA resistant if platelets are aggregating from a trigger other than TXA2, even though aspirin might be effectively inhibiting COX-1, its sole target. The studies that utilize ASA metabolites are a notable exception. Due to its simplicity, the most commonly utilized assay is the Platelet Function Analyzer-100 (PFA-100). However, Light Transmission Aggregometry (LTA) remains the "gold standard" for monitoring platelet function, despite being more demanding technically [5].

Bleeding time: The bleeding time test has been widely used in the diagnosis of haemostatic defects, principally thrombocytopenia, qualitative platelet defects, von Willebrand's disease, and vascular disorders. It is frequently used as a preoperative screening procedure. When vessels are injured or severed, platelets adhere to the exposed subendothelial lining of the vessel and to exposed collagen.

Following the adhesion phase, platelets begin to aggregate at the wound site, forming the primary platelet plug. The purpose of the bleeding time test is to provide a measure of platelet function in small vessel hemostasis. A bleeding time is the time required for bleeding to stop flowing from a standardized puncture on the patient's forearm. Prolonged bleeding can be caused by a decreased platelet count, abnormal platelet function or the ingestion of aspirin, ibuprofen or other NSAIDs. Anticoagulant therapy will also prolong a bleeding time.

The classical 4 methods used for BT tests are Ivy, Template, Modified Template and Duke. The normal range can vary depending on the method used but is typically between 2 and 9 minutes [41].

PFA 100: The PFA-100 System provides automated assessment for inherited, acquired, or drug-induced platelet dysfunction. It quickly and easily measures platelet plug formation in a small whole blood sample (800 µL) and reports a "closure time" in 5-8 minutes. It measures the process of primary hemostasis and aids

in the rapid detection of platelet dysfunction. Through high-shear flow technology, the system provides a realistic hemodynamic environment for the measurement of primary hemostasis [42].

Thromboxane Metabolites: A method for identifying aspirin resistance in a subject comprising: administering to the subject a first dose of aspirin; collecting a urine sample from the subject; determining an amount of two or more thromboxane A_2 metabolites in the urine sample; normalizing the amount of the two or more thromboxane A_2 metabolites to an amount of a standard protein in the subject's urine to obtain a first thromboxane A_2 metabolite/standard protein ratio; and comparing the first thromboxane A_2 metabolite/standard protein ratio to a threshold ratio value wherein a thromboxane A_2 metabolite /standard protein value below the threshold value indicates an absence of aspirin resistance in the subject [43].

Optical Platelet Aggregation: utilizes a modified spectrophotometer in which platelet-rich plasma is incubated, stirred, and evaluated as aggregating agents are added. It assesses optical density changes which are detected photoelectrically as platelets begin to aggregate. Whole blood (9 ml) was drawn within 24 hours of administration of the last dose of aspirin and tests were done within 4 hours of sampling. The sample was added to 1 ml of 3.8% trisodium citrate (pH=6.5) solution and centrifuged for 20 min at 20°C. The platelet-rich plasma was removed, and followed by centrifugation of remaining specimen to obtain platelet-deficient plasma.

Platelet count was adjusted to 2×108/ml with the spectrophotometer (absorption of 0.650 at 6.30 mm refers to the 2×108/µl). Platelet-rich plasma (400 µl) was taken in the aggregometer (whole blood Lumi Aggregometer-Chronolog Corp, USA). After 5 min of incubation, ADP (10 µm) and AA (0.5 mg/ml) was added to get the respective aggregation. Aggregation was monitored for 7-10 min. The inhibition of aggregation was compared with the control value. Another sample of 2 ml blood anticoagulated with EDTA was collected for hemoglobin and platelet count analysis.

Rapid Platelet Function Assay (RPFA). In this point of-care test, sampled capillary blood is run through a transparent fibrinogen-coated cartridge that contains platelet agonists, such as ARA, collagen, or epinephrine (Accumetrics Inc). As thrombus is formed on its surface, light transmission through the cartridge changes, provides an indirect measure of platelet activation [5].

Platelet Reactivity (PR): The PR test measures platelet activation triggered by a standardized vascular injury. Blood from a forearm vein is sampled in a standardized fashion and then mixed with either EDTA-buffer or EDTA-formaldehyde-buffer, respectively. While in the EDTA-buffer, the activated platelets are dissolved while they remain fixed in the EDTA-formaldehyde medium. Centrifugation causes only unattached, non-activated platelets to remain in the supernatant and a platelet counter determines the numbers in each supernatant. The measurement is inversely proportional to the number of unattached platelets [5].

Flow Cytometric Analysis. Flow cytometry has employed in recent studies to assess activation-dependent changes in the surface expression of platelet receptors. The study of platelet activation after stroke or transient ischemic attack in patients on aspirin and those who were aspirin-free is performed [44].

Using monoclonal antibodies, they assessed expression of GPIIb/IIIa (CD41); GPIb (CD42b); P-Selectin (CD62p); GPIIb/IIIa activity (PAC-1); platelet/endothelial cell adhesion molecule, PECAM-1 (CD31); vitronectin receptor (CD51/CD61); lysosome associated membrane protein, LAMP-3 (CD63), LAMP-1 (CD107a); platelet endothelial tetraspan antigen, PETA-3 (CD151); CD40-ligand (CD154); thrombospondin (CD36), and platelet-leukocyte aggregates (CD151, CD14). Thrombospondin, GPIIb/IIIa, P-Selectin, CD40-ligand, and platelet-monocyte aggregates were significantly lower in the aspirin-treated group. It is found little correlation between the PFA-100, platelet aggregometry, and GPIIb/IIIa flow cytometry in patients receiving various platelet regimens [45].

Techniques, Advantages & Limitations of Aspirin Resistance:

TECHNIQUES	ADVANTAGES	LIMITATIONS
Optical platelet-aggregation [46]	Widely available, Correlated with clinical events	Not specific, Uncertain sensitivity, Labor intensive, Operator and interpreter dependent
Semi-automated platelet – aggregometry (PFA-100, Ultegra RPFA) [46]	Simple, Rapid	Uncertain sensitivity, Uncertain correlation with clinical events
Skin bleeding time [46]	Only in vivo test, Simple, Widely available, Independent of coagulation disorders	Not specific, Not sensitive, Operator dependent, Limited reproducibility, Uncertain correlation with clinical events
Urinary thromboxane excretion [5]	Correlated with clinical events	Uncertain specificity, Uncertain sensitivity, Uncertain reproducibility, Not widely evaluated
Platelet reactivity [5]	Simple, standardized	Not ASA specific, Limited testing, Uncertain sensitivity
Ultegra (RPFA) [5]	Simple, rapid, inexpensive, Sensitive, Semi-automated, Correlates with LTA	Not ASA specific, Depends on von Willebrand factor and hematocrit

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, and 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 [47].

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.

Limitations of existing Research: Despite our increasing understanding of aspirin resistance, the existing research presents a number of limitations. There is no consensus on a definition of aspirin resistance and on whether the definition should be based on clinical outcomes, laboratory evidence, or both, and there are no criteria for distinguishing true resistance from treatment failure. Numerous tests with varying methodologies, sensitivities, and specificities (and also their own limitations) have been used to assess platelet aggregation. Therefore, there is no accepted uniform measure for screening aspirin resistance in the clinical setting.

The clearly elucidated biologic mechanisms for aspirin resistance are still unknown and probably multifactorial. Clinical outcome studies are limited by small sample sizes, varied definitions of aspirin resistance, and inadequate controlling of confounding variables by the study designs. The clinical application of aspirin resistance will require studies on larger populations that define antiplatelet resistance using consistent and reproducible assays and correlate the measurements with clinical outcomes that can be improved by alterations in antiplatelet strategy (e.g.,

increasing the dose of an antiplatelet agent, adding or substituting a second antiplatelet agent).

CONCLUSION: The current limitations in our understanding of aspirin resistance are due to the difficulties in assessing platelet function and aspirin resistance. A lack of consensus on a definition of aspirin resistance, variability on aspirin dosages between studies and the use of numerous platelet assessment techniques each with their own limitations, have all contributed to the variable reporting of aspirin resistance.

However, it is clear that clinical aspirin resistance is a significant problem. While aspirin resistance can be detected with current laboratory techniques, a test with high positive and negative predictive values for important clinical end points remains elusive. Developing a technique of platelet analysis that is affordable, rapid, widely available, easily performed and interpreted is a priority.

Future directions: Before aspirin resistance can be accepted as a valid entity worthy of screening and treatment, it will be necessary to develop a standardized definition and test of aspirin resistance. The laboratory measure of antiplatelet effect used to identify patients who are aspirin-resistant needs to be specific, reliable and valid, and must correlate independently, significantly and consistently with an increased risk of ischemic vascular events.

This requires large studies and adjustment for all known potential confounders (e.g., smoking, ethnicity, compliance with aspirin, adverse drug interactions). Over time, the definition of aspirin resistance may need to be refined to include genetic determinants (e.g., COX-1 polymorphisms) that mediate aspirin resistance and are associated with risk of ischemic vascular events. Because of the scope of cardiovascular disease and the widespread use of aspirin to prevent cardiovascular events, even a modest improvement in its effectiveness will potentially have an enormous impact on morbidity and mortality, preventing hundreds of thousands of events worldwide.

REFERENCES:

1. Jack DB. One hundred years of aspirin. Lancet 1997; 350: 437-9.
2. The Royal Society of London. An account of the success of the bark of the willow in the cure of agues. In a letter to the Right Honourable George Earl of Macclesfield, President of R. S. from the Rev. Mr. Edmund Stone, of Chipping-Norton in Oxfordshire. Philos Trans R Soc Lond 1763; 53: 195-200.
3. Sneader W. The discovery of aspirin: a reappraisal. BMJ 2000; 321: 1591-4.
4. Craven LL. Experiences with aspirin (Acetylsalicylic acid) in the nonspecific prophylaxis of coronary thrombosis. Mississippi Valley Medical Journal 1953; 75: 38-44.
5. Kasotakis G, Pipinos II, and Lynch TG. Current evidence and clinical implications of aspirin resistance. Journal of vascular surgery 2009; 50: 1500-10.
6. Vane JR: Inhibition of prostaglandin synthesis as a mechanism of action for aspirin like drugs. Nat New Biol 1971; 231: 232-235.
7. Second International Study of Infarct Survival Collaborative Group. Randomized trial of intravenous steptokinase, oral aspirin both or neither among 17,187 cases of suspected acute myocardial infarctions: ISIS-2. Lancet 1988; 2: 349–360.
8. Antithrombotic Trialists' Collaboration. Collaborative meta-analysis of randomized trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. British Medical Journal 2002; 324: 71–86.
9. Helgason CM, Bolin KM, Hoff JA, Winkler SR, Mangat A, Tortorice KL, et al. Development of aspirin resistance in persons with previous ischemic stroke. Stroke 1994; 25: 2331–2336.
10. Pappas JM, Westengard JC, Bull BS. Population variability in the effect of aspirin on platelet function. Implications for clinical trials and therapy. Arch Pathol Lab Med 1994; 118: 801–804.
11. Grotemeyer KH, Scharafinski HW, Husstedt IW. Two-year follow-up of aspirin responder and aspirin non responder. A pilot-study including 18 post-stroke patients. Thromb Res 1993; 71: 397–403.
12. Gum PA, Kottke-Marchant K, Poggio ED, Gurm H, Welsh PA, Brooks L, et al. Profile and prevalence of aspirin resistance in patients with cardiovascular disease. American Journal of Cardiology 2001; 88: 230–235.
13. Sadiq PA, Puri A, Dixit M, Ghatak A, Dwivedi SK, Narain VS, Saran RK, Puri VK. Profile and Prevalence of Aspirin Resistance in Indian Patients with Coronary Artery Disease. Indian Heart Journal 2005; 57: 658–661.
14. Gurbel PA, Bliden KP, Hayes KM, et al. Platelet activation in myocardial ischemi syndromes. Expert Review of Cardiovascular Therapy 2004; 2: 535- 545.
15. Gurbel PA, Becker RC, Mann KG, et al: Platelet function monitoring in patients with coronary artery disease. Journal of Amrican College of Cardiology 2007; 50: 1822-1834.
16. Gurbel PA, Bliden KP, Guyer K, et al. Platelet reactivity in patients and recurrent events post-stenting: results of the PREPARE POSTSTENTING Study. Journal of American College of Cardiology 2005; 46: 1820-1826.
17. Gurbel PA, Tantry US. The rationale for and comparisons of different antiplatelet treatments in acute coronary syndrome. J Interv Cardiol 2008; 21(Suppl 1): S10-S17,
18. Udaya S. Tantry US, Mahla E, Paul A. Gurbel PA: Aspirin Resistance. Progress in Cardiovascular Diseases 2009; 52: 141–152.
19. Roth GJ, Stanford N, Majerus PW. Acetylation of prostaglandin synthase by aspirin. Proc Natl Acad Sci U S A 1975; 72: 3073-6.
20. Loll PJ, Picot D, Garavito RM. The structural basis of aspirin activity inferred from the crystal structure of inactivated prostaglandin H2 synthase. Nature Structural & Molecular Biology 1995; 2: 637-43.

21. Cipollone F, Patrignani P, Greco A, et al. Differential suppression of thromboxane biosynthesis by indobufen and aspirin in patients with unstable angina. Circulation 1997; 96: 1109-16.
22. Patrono C, Coller B, FitzGerald GA, Hirsh J, Roth G. Platelet-active drugs: the relationships among dose, effectiveness, and side effects: the Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. Chest 2004; 126: Suppl: 234S-264S.
23. Patrono C, García Rodríguez LA, Landolfi R and Baigent C: Low-Dose Aspirin for the Prevention of Atherothrombosis. B.Ch.N Engl J Med 2005; 353: 2373-83.
24. Patrono C, Coller B, Dalen JE, FitzGerald GA, Fuster V, Gent M, Hirsch J, Roth J. Platelet-active drugs: the relationships among dose, effectiveness, and side effects. Chest 2001; 119(suppl): 39S– 63S.
25. Weber AA, Przytulski B, Schanz A, Hohlfeld T, Schror K. Towards a definition of aspirin resistance: a typological approach. Platelets 2002; 13: 37–40.
26. Alexander JH, Harrington RA, Tuttle RH, Berdan LG, Lincoff AM, Deckers JW, Simoons ML, Guerci A, Hochman JS, Wilcox RG, et al, on behalf of the PURSUIT Investigators. Prior aspirin use predicts worse outcomes in patients with non-ST-elevation acute coronary syndromes. American Journal of Cardiology 1999; 83: 1147–1151.
27. 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.
28. Cheng X, Chen W, and Simon DI: Aspirin Resistance or Variable Response or Both? American Journal of Cardiology 2006;98[suppl]:11N–17N
29. Weber AA, Przytulski B, Schanz A, Hohlfeld T, Schror K. Towards a definition of aspirin resistance: a typological approach. Platelets 2002; 13:37–40.
30. Kawasaki T, Ozeki Y, Igawa T, Kambayashi J. Increased platelet sensitivity to collagen in individuals resistant to low-dose aspirin. Stroke 2000; 31: 591–595.
31. Zimmermann N et al. Aspirin resistance after coronary artery bypasses grafting. The Journal of Thoracic and Cardiovascular Surgery 2001; 121: 982–984.
32. Bhatt DL: Aspirin Resistance: More Than Just a Laboratory Curiosity. Journal of the American College of Cardiology 2004; 43(6): 1127-29.
33. FitzGerald GA. Parsing an enigma: the pharmacodynamics of aspirin resistance. Lancet 2003; 361: 542-44.
34. Schafer AI. Genetic and Acquired Determinants of Individual Variability of Response to Antiplatelet Drugs. Circulation 2003; 108: 910-11.
35. Schror K, Weber AA, Hohlfeld T. Aspirin "resistance". Blood Cells Mol Dis 2006; 7: 12-15.
36. Marco Cattaneo. Aspirin and clopidogrel resistance: clinical issues. Arterio throm Vas Biol 2004; 24 : 1980-84.
37. Eikelboom JW, Hirsh J, Weitz J et al. Aspirin resistant thromboxane biosynthesis and the risk of myocardial infarction, stroke, or cardiovascular death in patients at high risk for cardiovascular events. 2002; 105 : 1650-55.
38. Hankey GJ, Eikelboom JW. Aspirin resistance. Lancet 2006; 367 : 606-17.
39. Cotter G, Shemesh E, Zehavi M et al. Lack of aspirin effect: aspirin resistance or resistance to taking aspirin? American Heart Journal 2004; 147: 293-300.
40. Kour D, Tandon VR, Kapoor B, Mahajan A, Parihar A, Smotra S: Aspirin Resistance. Jk Science 2006;8(2): 116-17.
41. Burns ER, Lawrence C. Bleeding time. A guide to its diagnostic and clinical utility. Arch. Pathol. Lab. Med.1989; 113: 1219-1224.
42. Lippi G, Manzato F, Franchini M, et al. Establishment of reference values for the PFA-100 System platelet function analyzer in pediatrics. Clin Exp Med 2001; 1: 69-70.
43. Wong S, Appleberg M, Ward CM, Lewis DR. Aspirin Resistance in Cardiovascular Disease. Eur J Vasc Endovasc Surg. 2004; 27, 456–465.
44. Serebruany VL, Malinin AI, Oshrine BR, Sane DC, Takserman A, Atar D, Hennekens CH. Lack of uniform platelet activation in patients after ischemic stroke and choice of antiplatelet therapy. Thromb Res 2004; 113:197-204.
45. Hezard N, Metz D, Nazeyrollas P, Droulle C, Potron G, Nguyen P. PFA-100 and flow cytometry: can they challenge aggregometry to assess antiplatelet agents, other than GPIIbIIIa blockers, in coronary angioplasty? Thromb Res 2002; 108: 43-7.
46. Hankey GJ, Eikelboom JW: Aspirin resistance: May be a cause of recurrent ischemic vascular events in patients taking aspirin. British Medical Journal 2004; 328: 477-78.
47. 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. The New England Journal of Medicine 2006; 354(16): 1706–1717.

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