

Resistance to oral antiplatelet drugs – a Position Paper of the Working Group on antiplatelet drug resistance appointed by the Section of Cardiovascular Interventions of the Polish Cardiac Society

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Kardiol Pol 2008; 66: 470–476

Oral antiplatelet drugs are one of the cornerstones of the modern pharmacotherapy of cardiovascular diseases. Many randomised trials have proved the efficacy of acetylsalicylic acid (ASA) and clopidogrel in lowering the risk of adverse events in coronary artery disease patients [1-4].

ASA is a nonselective cyclooxygenase inhibitor. At doses used in cardiology it efficiently blocks its constitutive isoenzyme (COX-1) present in blood platelets. ASA acetylates serine in the amino acid chain near the enzyme active site, preventing contact with arachidonic acid and hence stopping thromboxane A₂ synthesis. The effect is irreversible during platelet life span in the circulation and lasts from 7 to 10 days. After oral intake ASA is absorbed mainly in the stomach, reaching its peak concentration in the blood after approximately 30 minutes. It is metabolised by esterases present in the blood and liver, causing its half-life to be around 15 minutes [5]. For these reasons ASA exerts its action mainly in the portal circulation, before reaching the liver. It was recently shown that enteric coated formulation ASA is absorbed worse in a moderately acidic intestinal environment and provokes a lower antiplatelet effect than plain formulation [6].

The second oral antiplatelet drug, clopidogrel, is a prodrug. Its active form, thiol derivative, is developed through oxidation to 2-oxy-clopidogrel and hydrolysis

– a process dependent mainly on cytochrome P450 isoenzymes CYP3A4 and CYP2B6. The drug's active form binds selectively and irreversibly with P2Y₁₂ receptor on the platelet membrane external surface, blocking it from interaction with ADP. Likewise for ASA, clopidogrel platelet receptor occupancy lasts for platelet life span: 7-10 days [7].

Oral antiplatelet drugs in secondary prevention lower the risk of further myocardial infarction by about 25% and death by 20% [8]. Nevertheless, in about 15% of patients with diagnosed ischaemic heart disease while on antiplatelet treatment in a one-year period there is re-hospitalisation due to disease aggravation, myocardial infarction or stroke, and there can also be cardiovascular death. This percentage of events reaches 27% if in one patient there is atherosclerotic involvement of three vascular beds: coronary, carotid and lower limbs [9].

It should be pointed out that the process of atherothrombosis is highly complicated and blockade of only one of its pathways cannot completely abolish it. For this reason one cannot expect that antiplatelet drugs used even according to current guidelines in secondary prevention can save the patient from all atherothrombotic complications. The same applies to lipid lowering and antihypertensive drugs.

Regarding the two last groups of drugs we can easily monitor their effects through lipid level or blood pressure

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level measurement and according to these results modify the dose to obtain the therapeutic target. The estimation of antiplatelet action of known drugs has not reached standardisation status and rests mainly on research conducted from the end of the 20th century, including cardiovascular patients [10]. Various laboratory methods showed that in a certain percentage of patients despite ASA and/or clopidogrel use the predefined (different from one study to another) level of platelet inhibition is not achieved.

These results are understandable. The drug response often shows in a given population a normal distribution. At a certain dose the majority of patients respond to the drug; a small percentage respond more than average, while some do not respond properly. Together with the dose escalation the number of low responders decreases and number of drug adverse events rises in patients on the opposite side of the curve [11].

The ASA antiplatelet effect demands blockade of about 95% of platelet COX-1 activity. It can be obtained at a dose as small as 30 mg used chronically [5]. Doses currently used in cardiology are twice as high or higher and for this reason the level of laboratory measured incomplete response for ASA is rather small. In the case of clopidogrel the drug response curve has all the characteristics of a normal distribution [12], with higher antiplatelet effect at loading dose 600 mg and maintenance dose 150 mg a day [13].

The above-mentioned incomplete antiplatelet effect of ASA and clopidogrel has begun to be defined as aspirin and clopidogrel resistance [14]. From the start (and still today) the main problem with 'resistance' was the lack of a clear definition due to the lack of a standardised method of platelet function monitoring and also a lack of clear and widely accepted cut-off values of this activity classifying the patient as a responder or non-responder (resistant).

In subsequent studies the 'resistance' began to be divided into two entities: laboratory and clinical 'resistance'.

Clinical 'resistance' to oral antiplatelet drugs can be present when in a given patient a cardiovascular event occurred while on antiplatelet drugs.

Laboratory 'resistance' to oral antiplatelet drugs can be present when despite oral antiplatelet drugs use the in vitro platelet reactivity is not blocked properly.

It should also be stressed that those two 'definitions' are not identical: laboratory 'resistance' does not have to end in an ischaemic event, and having an ischaemic event while on antiplatelet drugs does not have to be confirmed with laboratory measures as 'resistance', although in a certain percentage of patients those two phenomena overlap [15].

In some recent publications there is a tendency to change the term 'resistance' to 'treatment failure', referring to a similar incomplete effect after antihypertensives or lipid lowering therapy [16]. Although the two latter clinical situations can easily be assessed, in the case of antiplatelet treatment failure it is not objectively possible at the current level of knowledge and it is inappropriate to identify every ischaemic event with 'treatment failure' or 'clinical resistance'.

The opinion of the Working Group

The definition of clinical resistance or treatment failure with respect to oral antiplatelet agents imprecisely links the presence of ischaemic events with inappropriate response to antiplatelet drugs. Accordingly we propose that the use of those designations in relation to clinical events be stopped.

'Laboratory resistance'

The description of all methods used to measure platelet reactivity in the laboratory exceeds the scope of the current article. They were presented in detail elsewhere [17-20]. We will restrict ourselves to presenting which of the available methods are, in the opinion of the panel, most precisely able to estimate the laboratory effect of ASA and clopidogrel. It is only a proposition which can be changed along with the presence of newer methods and better reproductivity of current ones.

The methodological problems were recently elegantly described in a study in which six different tests were compared in a group of patients with stable coronary artery disease. All patients were on chronic ASA therapy. The ASA 'resistance' was 'diagnosed' in from 6% to 60% of the group depending on the method [21]. Results hardly correlated with one another. Extreme caution is needed while dealing with in vitro platelet reactivity estimation after oral antiplatelet drugs.

ASA effect estimation

- A) The proposed test is platelet aggregation induced by arachidonic acid, a substrate of COX-1 blocked by ASA. It can be performed in whole blood using impedance aggregometry or the point-of-care VerifyNow[®] system or in platelet-rich plasma using optical aggregometry.
- B) For the above-mentioned methods investigators or device producers established certain cut-off values, which help to classify patients as good or low responders. At present the majority of such cut-off values relate to optical aggregation induced with arachidonic acid in a concentration of 0.5 mg/ml [22], or impedance aggregometry in whole blood with the same agonist [23]. A low response to ASA is defined as a value above **10-20%** [24, 25] in optical aggregation or above 0 Ω in impedance aggregation [23]. It should be stressed that so far there is no definitive proof that the given cut-off value is linked with worse cardiovascular outcome in the future. The search for such proof and maybe new cut-off values is under investigation.
- C) It seems that the most precise test for biochemical effect of ASA is the measurement of thromboxane B₂ level (stable metabolite of thromboxane A₂) in serum obtained after one hour from whole blood staying without anticoagulant at a temperature of 37°C. Other tests seem to measure other sources of thromboxane rather than

platelets (11-dihydro-thromboxane B₂ in urine) or are difficult to perform from the analytical point of view (thromboxane B₂ in plasma) [26, 27]. In the proposed method we currently also do not have clinically relevant cut-off values.

Clopidogrel effect estimation

- A) The proposed test is platelet aggregation induced by ADP. It can be performed in whole blood using impedance aggregometry or the point-of-care VerifyNow® system or in platelet-rich plasma using optical aggregometry.
- B) It seems that elevated risk of ischaemic events while on clopidogrel may be at least partially dependent on optical aggregation above **60%** (induced by ADP at a concentration of 20 μM) and/or optical aggregation above **50%** (induced by ADP at a concentration of 5 μM). There is no need to estimate the relative change in aggregation level before and after clopidogrel use [28]. Those cut-off values can be taken into consideration for further verification in large clinical trials.
- C) Another method for clopidogrel effect estimation is flow cytometric analysis of VASP protein phosphorylation [29]. It is an expensive and demanding method but at the same time seems to be more precise from the mechanistic point of view to track and explore the specific pathway of platelet activation through the P2Y₁₂ receptor. The cut-off value linked to increased risk of cardiovascular events proposed by some investigators is **PRI (platelet reactivity index) >53** [30]. It needs to be confirmed in other studies.

Nonspecific methods of platelet reactivity measurement

Other methods for monitoring effects of antiplatelet agents are nonspecific. They are point-of-care PFA-100®, Impact cone and plat(elet)® and optical and impedance aggregometry using agonists other than arachidonic acid and ADP (collagen, thrombin and others). Results obtained with these devices describe platelet reactivity while on treatment, and only by far approximation can they be attributed to the specific pathways blocked by ASA and clopidogrel [31].

Pharmacodynamic vs pharmacokinetic ‘resistance’

The lack of a standardised laboratory method for platelet reactivity explains the lack of a proper definition of the whole ‘resistance’ phenomenon. For some time now there have been propositions to make this definition precise at least for ASA. The ‘resistance’ was divided into pharmacodynamic, pharmacokinetic and pseudo resistance [31, 32]. The principle lies in performance of a functional exam (aggregation) and biochemical one (thromboxane B₂ concentration in serum or in supernatant obtained after aggregation). For clopidogrel there is no such opinion.

Pharmacodynamic ASA ‘resistance’. It can be provoked by a change in the target enzyme for ASA – COX-1:

a change in enzyme conformation (gene polymorphism) [33, 34] or its transient inaccessibility due to blockade of the active site by a nonsteroidal anti-inflammatory agent (ibuprofen) [35]. In that case in vitro addition of ASA to the blood sample would not change aggregation level significantly, nor would it influence thromboxane B₂ level [31, 32].

Pharmacodynamic clopidogrel ‘resistance’. It can be provoked by a change in the target receptor – P2Y₁₂ (gene polymorphism), although available reports seem not to confirm that idea [30]. It is more difficult to perform in vitro a similar experiment like with ASA due to limited availability of the active drug form, though another P2Y₁₂ receptor antagonist can be considered [36]. For the proper ‘diagnosis’ it is also strongly recommended (though difficult to perform) to measure the level of the active form of clopidogrel in blood to prove its good bioavailability.

Pharmacokinetic ASA ‘resistance’. The main reason for it is the limited availability of the active drug at its target. Adding ASA in vitro to the blood sample should block or significantly reduce aggregation as well as thromboxane B₂ concentration [31, 32]. It can be induced by too low dose, change in drug absorption, transportation, use of enteric-coated formulation (less absorbed in intestine). One should also consider increased production of ‘young’, more active platelets, which cannot be blocked with once daily ASA in a relatively small dose [37].

Pharmacokinetic clopidogrel ‘resistance’. It can be induced by too small dose, change in its absorption and conversion to active drug in the liver (gene polymorphisms for respective enzymes in a chain of prodrug transformation) [38]. The reason can also lie, as in the case of ASA pharmacokinetic ‘resistance’, in elevated production of ‘young’ platelets with higher density of P2Y₁₂ receptors.

Apart from the above propositions of a systematic approach to the ‘resistance’ phenomenon there are other situations which can be classified as ‘pseudo resistance’: transient COX-2 expression in ‘young’ platelets, extra platelet sources of thromboxane or delivery to platelets of substrates for thromboxane production (from endothelial cells or monocytes) bypassing COX-1 blocked by ASA [39].

In the opinion of the Working Group laboratory ‘resistance’ should be better identified as pharmacodynamic ‘resistance’, where increased platelet reactivity is due to an improper blockade of the target enzyme (as to ASA) or receptor (as to clopidogrel).

The opinion of the Working Group – for research purposes only

1. Laboratory ‘resistance’ for oral antiplatelet agents should be referred to the situation when the proper effect cannot be obtained due to changes in a target enzyme or receptor (pharmacodynamic ‘resistance’). It is possible to detect it with a certain approximation in vitro.

2. For ASA effect estimation it is proposed to use aggregation induced by arachidonic acid and thromboxane B₂ concentration in serum or in the supernatant after aggregation with the possible *in vitro* addition of ASA.
3. For clopidogrel effect estimation it is proposed to perform aggregation induced with ADP (and possible *in vitro* addition of P2Y₁₂ antagonist) or VASP phosphorylation.
4. In the case of abnormal results of nonspecific tests one can only talk about 'elevated platelet reactivity despite treatment'. To detect the reason for this, more specific tests for a given drug should be performed.

The extent of the 'resistance' phenomenon

The frequency of clopidogrel 'resistance', investigated mainly using optical aggregation induced by ADP, ranges in the available data from 5 to 44% [40, 41]. This discrepancy reflects the imperfection of currently available laboratory tests, its incompatibility, possible pre-analytical and analytical errors, different groups studied as well as different research protocols and predefined cut-off values.

The same holds for ASA 'resistance', which according to a recent meta-analysis is present on average in 27.1% of patients (95% CI 21.5-32.6%), although available data report the frequency of the phenomenon as from 0% to as high as 57% [42]. This discrepancy may be due, as in clopidogrel, to different agonists, laboratory methods, and ASA 'resistance' definitions. They may also be due to different drug doses; a chronic dose of 300mg ASA can overcome the 'resistance' in some subjects, showing that the reason for this may reside in the pharmacokinetics of the drug. The same is true for clopidogrel dose.

The above-mentioned frequency of ASA resistance is obtained from different studies implementing different laboratory protocols. Choosing those with aggregation induced with arachidonic acid and/or thromboxane serum level gives an average frequency of the phenomenon of 6% (95% CI 0-12%) [42].

The opinion of the Working Group

The exact estimation of 'resistance' frequency is at present impossible. This is mainly because we do not have a definition or an established laboratory method. The current position paper hopes to put in order future research in that field to obtain reliable laboratory data to resolve the problem.

Antiplatelet treatment monitoring

The practitioner meets every day patients with cardiovascular events due to atherothrombosis. A special category includes rare but severe cases of stent

thrombosis, more frequent, as was shown recently, in patients with a drug-eluting stent implanted [43]. One has to keep in mind though that platelets are not the only reason for those events [44, 45].

Methodological problems of the evaluation of platelet reactivity puts antiplatelet drug effect monitoring in an early stage of standardisation for daily clinical practice. This concern is also seen in the European Society of Cardiology guidelines, which do not recommend routine use of such monitoring [46].

The American societies (AHA/ACC) went a step further [47], asserting that clopidogrel antiplatelet activity can be considered in patients in whom possible stent thrombosis could lead to devastating complications. This category comprises patients after unprotected left main stenting, left main bifurcation or last patent coronary vessel stenting (Class IIb, Level of Evidence: C) According to those guidelines one should consider doubling the chronic clopidogrel dose to 150 mg/day in patients with less than 50% blockade of platelet aggregation. At the same time there is no recommendation for ASA 'resistance'.

It should be noted that the American guidelines do not precisely define the method of platelet reactivity evaluation and for this reason are not practically possible to implement. There is no information about the method of aggregation, agonist and its concentration. There is also no indication whether the 50% value relates to absolute aggregation or relative aggregation after comparing it to the value before drug intake.

The opinion of the Working Group

At this point in time there are no clinical data obtained from randomised trials showing that there is an advantage and expediency for routine determination of antiplatelet drugs effect.

Proceeding in situations with incomplete response to antiplatelet drugs

Similarly, there are no recommendations for 'treating' a potential incomplete response 'discovered' with monitoring. Nevertheless, some investigators propose considering the use of GP IIb/IIIa as an additional treatment during elective angioplasty in such 'resistant' patients [48], while others suggest increasing clopidogrel dose up to 150 mg for chronic use [49]. One can also consider cilostazol as a third antiplatelet drug [50]. New antiplatelet drugs could be of additional value in such situations. Those drugs are currently under investigation. Recent clinical data show that the new thienopyridine derivative (prasugrel) exerts more antiplatelet action than clopidogrel, which indirectly may be connected with fewer stent thrombotic events, although the risk of severe bleeding while on prasugrel also rises [51]. All those situations and propositions should be tested in randomised, prospective trials.

The 'increased platelet reactivity while on treatment' cannot be ignored, after all. In certain situations and in certain groups of patients it is connected with poor prognosis [52]. It was proved in patients on ASA and clopidogrel with specific and non-specific point-of-care methods [53-58].

It should also be noted that an incomplete response to antiplatelet drugs was shown in diabetics [59], obese patients [60, 61], in cases of hypercholesterolaemia [62] and in smokers [63]. Taking this into account, it is worth intensively modifying those risk factors with a potential influence on the action of antiplatelet drugs as well.

In vitro platelet reactivity is also closely connected with individual compliance. The percentage of non-compliant patients, as for ASA and clopidogrel, could be as high as 18% [64]. At every contact with the patients it is essential to check/remind them of drug taking, because paradoxically the most common cause of 'resistance' could be 'resistance to taking' ASA or clopidogrel.

The European Society of Cardiology recommends for chronic use, ASA in doses 75-150 mg and clopidogrel 75-100 mg/day. These recommendations derive from analysis of the CURE trial [65], as well as from the Antiplatelet Trialists' Collaboration meta-analysis [8], where it was shown that ASA doses mentioned bring the most advances with fewer risks of bleeding complications. From the other point of view the less ASA 'resistant' patients are in groups where the ASA dose is higher than those recommended [42]. For this reason in currently ongoing trials higher doses of ASA and clopidogrel are being evaluated. Those trials (ASCET [66], GRAVITAS, CURRENT, RESISTOR) are designed to monitor the antiplatelet effect of ASA and clopidogrel or simply increase the dose without in vitro platelet reactivity evaluation. The main combined aim of those trials is to investigate whether increasing antiplatelet agent dose would improve the clinical outcome, especially in the group of 'resistant' patients.

The opinion of the Working Group

1. Currently there are no recommendations for chronic monitoring of antiplatelet drug activity in clinical practice, or dose modification in case of 'low response'.
2. When there is a suspicion of 'resistance' it should be confirmed whether the patients is compliant with recommended drugs.
3. In academic centers with experience in platelet reactivity tests individual antiplatelet dosing could be implemented in strictly individual cases [e.g. patients with multiple cardiovascular risk factors and recurrent thrombotic events (stent thrombosis) despite compliance with standard antiplatelet drug doses]. Those actions could be undertaken as a research study or on a casuistic basis.

Perspectives – authors' point of view

At the current stage of research it seems that more important than classifying patients as 'responders' or 'non-responders' is to set up cut-off values for platelet reactivity connected with worse/better clinical outcome. Such an approach could also clarify the sensitivity and specificity of each of the laboratory methods, which hopefully would lead to clinical use of the best one in the future.

Currently ongoing research apart from cut-off values could also indicate certain groups of patients that could profit from drug effect monitoring. It should go with the cost-effectiveness of platelet reactivity tests. At the time being those groups could include diabetics, patients after cerebrovascular incidents, with peripheral artery diseases, after coronary artery bypass grafting and with history of stent thrombosis.

References

1. Randomised trial of intravenous streptokinase, oral aspirin, both, or neither among 17,187 cases of suspected acute myocardial infarction: ISIS-2. ISIS-2 (Second International Study of Infarct Survival) Collaborative Group. *Lancet* 1988; 2: 349-60.
2. A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE). CAPRIE Steering Committee. *Lancet* 1996; 348: 1329-39.
3. Yusuf S, Zhao F, Mehta SR, et al. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. *N Engl J Med* 2001; 345: 494-502.
4. Chen ZM, Jiang LX, Chen YP, et al. Addition of clopidogrel to aspirin in 45,852 patients with acute myocardial infarction: randomised placebo-controlled trial. *Lancet* 2005; 366: 1607-21.
5. Patrono C, Collier B, Dalen JE, et al. Platelet-active drugs: the relationships among dose, effectiveness, and side effects. *Chest* 2001; 119: 39S-63S.
6. Cox D, Maree AO, Dooley M, et al. Effect of enteric coating on antiplatelet activity of low-dose aspirin in healthy volunteers. *Stroke* 2006; 37: 2153-8.
7. Quinn MJ, Fitzgerald DJ. Ticlopidine and clopidogrel. *Circulation* 1999; 100: 1667-72.
8. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. *BMJ* 2002; 324: 71-86.
9. Steg PG, Bhatt DL, Wilson PW, et al. One-year cardiovascular event rates in outpatients with atherothrombosis. *JAMA* 2007; 297: 1197-206.
10. Tantry US, Bliden KP, Gurbel PA. Resistance to antiplatelet drugs: current status and future research. *Expert Opin Pharmacother* 2005; 6: 2027-45.
11. Wilkinson GR. Drug metabolism and variability among patients in drug response. *N Engl J Med* 2005; 352: 2211-21.
12. Serebruany VL, Steinhubl SR, Berger PB, et al. Variability in platelet responsiveness to clopidogrel among 544 individuals. *J Am Coll Cardiol* 2005; 45: 246-51.
13. Gurbel PA, Tantry US. Drug insight: Clopidogrel nonresponsiveness. *Nat Clin Pract Cardiovasc Med* 2006; 3: 387-95.
14. Gurbel PA, Tantry US. Aspirin and clopidogrel resistance: consideration and management. *J Interv Cardiol* 2006; 19: 439-48.

15. Snoep JD, Hovens MM, Eikenboom JC, et al. Association of laboratory-defined aspirin resistance with a higher risk of recurrent cardiovascular events: a systematic review and meta-analysis. *Arch Intern Med* 2007; 167: 1593-9.
16. Cattaneo M. Resistance to antiplatelet drugs: molecular mechanisms and laboratory detection. *J Thromb Haemost* 2007; 5 Suppl 1: 230-7.
17. Harrison P, Frelinger AL 3rd, Furman MI, et al. Measuring antiplatelet drug effects in the laboratory. *Thromb Res* 2007; 120: 323-36.
18. Chakroun T, Addad F, Abderazek F, et al. Screening for aspirin resistance in stable coronary artery patients by three different tests. *Thromb Res* 2007.
19. Cox D, Fitzgerald D. Monitoring antiplatelet therapy. In: Gesele P, Page CP, Fuster V, Vermylen J (eds.). *Platelets in Thrombotic and Non-thrombotic Disorders*. Cambridge University Press; Cambridge 2002: 471-84.
20. Matzdorff A. Platelet function tests and flow cytometry to monitor antiplatelet therapy. *Semin Thromb Hemost* 2005; 31: 393-9.
21. Lordkipanidze M, Pharand C, Schampaert E, et al. A comparison of six major platelet function tests to determine the prevalence of aspirin resistance in patients with stable coronary artery disease. *Eur Heart J* 2007; 28: 1702-8.
22. Gum PA, Kottke-Marchant K, Welsh PA, et al. A prospective, blinded determination of the natural history of aspirin resistance among stable patients with cardiovascular disease. *J Am Coll Cardiol* 2003; 41: 961-5.
23. Golanski J, Chlopicki S, Golanski R, et al. Resistance to aspirin in patients after coronary artery bypass grafting is transient: impact on the monitoring of aspirin antiplatelet therapy. *Ther Drug Monit* 2005; 27: 484-90.
24. Gum PA, Kottke-Marchant K, Poggio ED, et al. Profile and prevalence of aspirin resistance in patients with cardiovascular disease. *Am J Cardiol* 2001; 88: 230-5.
25. Kuliczowski W, Halawa B, Korolko B, et al. Aspirin resistance in ischaemic heart disease. *Kardiologia Pol* 2005; 62: 14-25.
26. Reilly IA, Doran JB, Smith B, et al. Increased thromboxane biosynthesis in a human preparation of platelet activation: biochemical and functional consequences of selective inhibition of thromboxane synthase. *Circulation* 1986; 73: 1300-9.
27. Patrono C, Ciabattoni G, Pinca E, et al. Low dose aspirin and inhibition of thromboxane B2 production in healthy subjects. *Thromb Res* 1980; 17: 317-27.
28. Gurbel PA, Bliden KP, Samara W, et al. Clopidogrel effect on platelet reactivity in patients with stent thrombosis: results of the CREST Study. *J Am Coll Cardiol* 2005; 46: 1827-32.
29. Aleil B, Ravanat C, Cazenave JP, et al. Flow cytometric analysis of intraplatelet VASP phosphorylation for the detection of clopidogrel resistance in patients with ischemic cardiovascular diseases. *J Thromb Haemost* 2005; 3: 85-92.
30. Frere C, Cuisset T, Quilici J, et al. ADP-induced platelet aggregation and platelet reactivity index VASP are good predictive markers for clinical outcomes in non-ST elevation acute coronary syndrome. *Thromb Haemost* 2007; 98: 838-43.
31. Weber AA, Przytulski B, Schanz A, et al. Towards a definition of aspirin resistance: a typological approach. *Platelets* 2002; 13: 37-40.
32. Pulcinelli FM, Riondino S. More on aspirin resistance: position paper of the Working Group on Aspirin Resistance. Proposal for a Laboratory Test Guiding Algorithm. *J Thromb Haemost* 2006; 4: 485-7.
33. Maree AO, Curtin RJ, Chubb A, et al. Cyclooxygenase-1 haplotype modulates platelet response to aspirin. *J Thromb Haemost* 2005; 3: 2340-5.
34. Goodman T, Sharma P, Ferro A. The genetics of aspirin resistance. *Int J Clin Pract* 2007; 61: 826-34.
35. Catella-Lawson F, Reilly MP, Kapoor SC, et al. Cyclooxygenase inhibitors and the antiplatelet effects of aspirin. *N Engl J Med* 2001; 345: 1809-17.
36. Kunapuli SP, Ding Z, Dorsam RT, et al. ADP receptors – targets for developing antithrombotic agents. *Curr Pharm Des* 2003; 9: 2303-16.
37. Zimmermann N, Wenk A, Kim U, et al. Functional and biochemical evaluation of platelet aspirin resistance after coronary artery bypass surgery. *Circulation* 2003; 108: 542-7.
38. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, et al. Contribution of gene sequence variations of the hepatic cytochrome P450 3A4 enzyme to variability in individual responsiveness to clopidogrel. *Arterioscler Thromb Vasc Biol* 2006; 26: 1895-900.
39. Tran HA, Anand SS, Hankey GJ, et al. Aspirin resistance. *Thromb Res* 2007; 120: 337-46.
40. Muller I, Besta F, Schulz C, et al. Prevalence of clopidogrel non-responders among patients with stable angina pectoris scheduled for elective coronary stent placement. *Thromb Haemost* 2003; 89: 783-7.
41. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, et al. Identification of low responders to a 300-mg clopidogrel loading dose in patients undergoing coronary stenting. *Thromb Res* 2005; 115: 101-8.
42. Hovens MM, Snoep JD, Eikenboom JC, et al. Prevalence of persistent platelet reactivity despite use of aspirin: a systematic review. *Am Heart J* 2007; 153: 175-81.
43. Bavry AA, Kumbhani DJ, Helton TJ, et al. Late thrombosis of drug-eluting stents: a meta-analysis of randomized clinical trials. *Am J Med* 2006; 119: 1056-61.
44. Iakovou I, Schmidt T, Bonizzoni E, et al. Incidence, predictors, and outcome of thrombosis after successful implantation of drug-eluting stents. *JAMA* 2005; 293: 2126-30.
45. Kuchulakanti PK, Chu WW, Torguson R, et al. Correlates and long-term outcomes of angiographically proven stent thrombosis with sirolimus- and paclitaxel-eluting stents. *Circulation* 2006; 113: 1108-13.
46. Silber S, Albertsson P, Aviles FF, et al. Guidelines for percutaneous coronary interventions. The Task Force for Percutaneous Coronary Interventions of the European Society of Cardiology. *Eur Heart J* 2005; 26: 804-47.
47. Smith SC Jr, Feldman TE, Hirshfeld JW Jr, et al. ACC/AHA/SCAI 2005 guideline update for percutaneous coronary intervention a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (ACC/AHA/SCAI Writing Committee to Update the 2001 Guidelines for Percutaneous Coronary Intervention). *J Am Coll Cardiol* 2006; 47: e1-121.
48. Bliden KP, DiChiara J, Tantry US, Bassi AK, Chaganti SK, Gurbel PA. Increased risk in patients with high platelet aggregation receiving chronic clopidogrel therapy undergoing percutaneous coronary intervention: is the current antiplatelet therapy adequate? *J Am Coll Cardiol* 2007; 49: 657-66.
49. von Beckerath N, Kastrati A, Wieczorek A, et al. A double-blind, randomized study on platelet aggregation in patients treated with a daily dose of 150 or 75 mg of clopidogrel for 30 days. *Eur Heart J* 2007; 28: 1814-9.
50. Kim JY, Lee K, Shin M, et al. Cilostazol could ameliorate platelet responsiveness to clopidogrel in patients undergoing primary percutaneous coronary intervention. *Circ J* 2007; 71: 1867-72.

51. Wiviott SD, Braunwald E, McCabe CH, et al. Prasugrel versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med* 2007; 357: 2001-15.
52. Huczek Z, Filipiak KJ, Kochman J, et al. Baseline platelet reactivity in acute myocardial infarction treated with primary angioplasty – influence on myocardial reperfusion, left ventricular performance, and clinical events. *Am Heart J* 2007; 154: 62-70.
53. Cuisset T, Frere C, Quilici J, et al. High post-treatment platelet reactivity identified low-responders to dual antiplatelet therapy at increased risk of recurrent cardiovascular events after stenting for acute coronary syndrome. *J Thromb Haemost* 2006; 4: 542-9.
54. Cuisset T, Frere C, Quilici J, et al. High post-treatment platelet reactivity is associated with a high incidence of myonecrosis after stenting for non-ST elevation acute coronary syndromes. *Thromb Haemost* 2007; 97: 282-7.
55. Geisler T, Langer H, Wydymus M, et al. Low response to clopidogrel is associated with cardiovascular outcome after coronary stent implantation. *Eur Heart J* 2006; 27: 2420-5.
56. Gurbel PA, Bliden KP, Hayes KM, et al. The relation of dosing to clopidogrel responsiveness and the incidence of high post-treatment platelet aggregation in patients undergoing coronary stenting. *J Am Coll Cardiol* 2005; 45: 1392-6.
57. Hochholzer W, Trenk D, Bestehorn HP, et al. Impact of the degree of peri-interventional platelet inhibition after loading with clopidogrel on early clinical outcome of elective coronary stent placement. *J Am Coll Cardiol* 2006; 48: 1742-50.
58. Marcucci R, Panizza R, Antonucci E, et al. Residual platelet reactivity is an independent predictor of myocardial injury in acute myocardial infarction patients on antiaggregant therapy. *Thromb Haemost* 2007; 98: 844-51.
59. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, et al. Platelet function profiles in patients with type 2 diabetes and coronary artery disease on combined aspirin and clopidogrel treatment. *Diabetes* 2005; 54: 2430-5.
60. Feher G, Koltai K, Alkonyi B, et al. Clopidogrel resistance: role of body mass and concomitant medications. *Int J Cardiol* 2007; 120: 188-92.
61. Tamminen M, Lassila R, Westerbacka J, et al. Obesity is associated with impaired platelet-inhibitory effect of acetylsalicylic acid in nondiabetic subjects. *Int J Obes Relat Metab Disord* 2003; 27: 907-11.
62. Markuszewski L, Rosiak M, Golanski J, et al. Reduced blood platelet sensitivity to aspirin in coronary artery disease: are dyslipidaemia and inflammatory states possible factors predisposing to sub-optimal platelet response to aspirin? *Basic Clin Pharmacol Toxicol* 2006; 98: 503-9.
63. Mirkhel A, Peyster E, Sundeen J, et al. Frequency of aspirin resistance in a community hospital. *Am J Cardiol* 2006; 98: 577-9.
64. Kulkarni SP, Alexander KP, Lytle B, et al. Long-term adherence with cardiovascular drug regimens. *Am Heart J* 2006; 151: 185-91.
65. Fox KA, Mehta SR, Peters R, et al. Benefits and risks of the combination of clopidogrel and aspirin in patients undergoing surgical revascularization for non-ST-elevation acute coronary syndrome: the Clopidogrel in Unstable angina to prevent Recurrent ischemic Events (CURE) Trial. *Circulation* 2004; 110: 1202-8.
66. Pettersen AA, Seljeflot I, Abdelnoor M, et al. Unstable angina, stroke, myocardial infarction and death in aspirin non-responders. A prospective, randomized trial. The ASCET (ASpirin non-responsiveness and Clopidogrel Endpoint Trial) design. *Scand Cardiovasc J* 2004; 38: 353-6.