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SPECIAL ARTICLE

Interindividual variability in the response to oral antiplatelet drugs: a position paper of the Working Group on antiplatelet drugs resistance appointed by the Section of Cardiovascular Interventions of the Polish Cardiac Society, endorsed by the Working Group on Thrombosis of the European Society of Cardiology

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Oral antiplatelet drugs are a cornerstone of modern pharmacotherapy in cardiovascular atherothrombotic diseases. The efficacy of acetylsalicylic acid (ASA, aspirin) and clopidogrel in decreasing the risk of adverse events in coronary heart disease patients has been well established in the past 20 years. Despite chronic oral antiplatelet therapy, a number of atherothombotic events continue to occur. In recent years, a number of reports in the literature have shown possible relationships between residual platelet activity, as measured with a variety of laboratory tests, and clinical outcome, raising the possibility that 'resistance' to oral antiplatelet drugs may underlie many such clinical adverse events. The present position paper, conveyed within a group of clinical cardiologists with expertise in thrombosis appointed by the Section of Cardiovascular Interventions of the Polish Cardiac Society, has been further elaborated and endorsed by the Working Group on Thrombosis of the European Society of Cardiology. It aims at summarizing the main findings in this complex area, issuing opinions in cases of high controversy, and fostering future research in this area to obtain reliable laboratory and clinical data for the resolution of the many problems still open.

Keywords Platelets • Antiplatelet agents • Monitoring • Resistance • Variability • Aspirin • Clopidogrel

Preamble: aims and scope

Oral antiplatelet drugs are a cornerstone of modern pharmacotherapy in cardiovascular atherothrombotic diseases. The efficacy of acetylsalicylic acid (ASA, aspirin) and clopidogrel in decreasing the risk of adverse events in coronary heart disease (CHD) patients[1–4] has been well established in the past 20 years. Despite chronic oral antiplatelet therapy, a number of

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atherothombotic events continue to occur. In recent years, a number of reports in the literature have shown possible relationships between residual platelet activity, as measured with a variety of laboratory tests, and clinical outcome, raising the possibility that 'resistance' to oral antiplatelet drugs may underlie many such clinical adverse events.

The present position paper, conveyed within a group of clinical cardiologists with expertise in thrombosis appointed by the Section of Cardiovascular Interventions of the Polish Cardiac Society, and published in a previous version in the Polish Cardiology Journal,[5] has been further elaborated and endorsed by the Working Group on Thrombosis of the European Society of Cardiology. This position paper aims at summarizing the main findings in this complex area, issuing opinions in cases of high controversy, and fostering future research in this area to obtain reliable laboratory and clinical data for the resolution of the many problems still open.

Background

ASA is a non-selective cyclooxygenase inhibitor. At doses used in cardiology, ASA efficiently blocks the constitutive isoenzyme cyclooxygenase (COX)-1 present in blood platelets. ASA acetylates a serine in the amino acid chain near the enzyme active site, thus preventing the contact with arachidonic acid and hence stopping thromboxane (TX) A_2 synthesis. The effect is irreversible throughout the entire life span of platelet in the circulation, and therefore lasts from 7 to 10 days. Moreover, ASA blocks exclusively one amplification pathway of platelet activation, while many other pathways stimulated by adenosine diphosphate (ADP), adrenaline, thrombin, shear stress, and high-dose collagen remain largely unaffected.

After oral intake, ASA is absorbed mainly in the stomach, reaching its peak concentration in blood after ~30 min. It is metabolized by esterases present in the blood and in the liver, explaining its short half-time, in the order of 15 min.[6] For this reason, ASA exerts its action mainly in the portal circulation, before reaching the liver. It has recently been shown that enteric-coated formulations of ASA are absorbed worse than standard plain formulations in the moderately acidic intestinal environment, and provoke a lesser antiplatelet effect.[7]

A second oral antiplatelet drug, clopidogrel, is a pro-drug, and its active form, a thiol derivative, is produced through oxidation to 2-oxy-clopidogrel and hydrolysis, in a process mainly dependent on the cytochrome P450 isoenzymes 3A4, 3A5, 2B6, and 2C19 and, to a lesser extent, on isoenzymes 1A1 and 1A2. The active form of the drug binds selectively and irreversibly to the $P2Y_{12}$ receptor on the external surface of the platelet membrane, blocking the interaction of the receptor with ADP. As for ASA, clopidogrel platelet receptor occupancy lasts for the entire life span of platelet, i.e. for 7–10 days.[8]

Oral antiplatelet drugs in secondary prevention decrease the risk of a subsequent myocardial infarction by about 25% and death by 20%.[9] Despite the use of such agents, there is a residual rate of re-hospitalization in about 15% of patients with diagnosed ischaemic heart disease while on antiplatelet treatment, due to worsening of the disease or the occurrence of myocardial infarction, stroke and cardiovascular death. This percentage of events reaches 27% in a 1 year time if there is atherosclerotic involvement of three vascular beds—the coronary arteries, the carotid arteries, and arteries of the lower limbs.[10] The issue has therefore arisen whether the occurrence of largely atherothrombotic events is due to insufficient inhibition of platelet function in some specific subjects or disease settings.

It should be at first pointed out that atherothrombosis is a complex process, and the blockade of only one of its mechanisms—platelet function—albeit important, cannot completely abolish the entire process and its consequences. For this reason, one cannot expect that antiplatelet drugs, despite their careful use according to current guidelines in secondary prevention, can save all patients from all atherothrombotic complications. The same happens for lipid-lowering and blood pressure-lowering drugs. We broadly define such cardiovascular events as 'treatment failures', without the implication of a cause–effect relationship between the inappropriate/insufficient use of the drug and ensuing clinical events.

However, for lipid-lowering and blood pressure-lowering drugs, we can easily monitor their effects through measurements of the intermediate endpoints of lipid levels or blood pressure, and we can accordingly modify/titrate the dose of the drug to achieve the optimal control of the intermediate therapeutic target. Measurement of the action of antiplatelet drugs has not reached such a status of standardization and is still a research tool after more 30 years of intense work.[11]

Interindividual variability of antiplatelet agents

Various laboratory methods have shown that, in a certain percentage of patients, a predefined level of platelet inhibition (different between studies) is not achieved despite the use of ASA and/or clopidogrel. Such results are understandable, as the drug response tested in a group of healthy individuals or patients mostly shows a normal distribution:[12] at a certain given dose, the majority of patients will respond to the drug, a small percentage will respond more than the average, and some patients will respond less. The number of low-responders will usually decrease (but drug-related adverse events will also somewhat increase) hand-in-hand with a dose escalation.[13]

The antiplatelet effect of ASA, however, requires a blockade of about 95% of platelet COX-1 activity. This can be obtained already at a dose as small as 30 mg used chronically.[6] No further increase in antiplatelet potency of ASA can be expected with increasing doses above 80–100 mg because a more than complete inhibition of COX-1-dependent platelet TX formation is not possible and because the COX-2-dependent thromboxane formation in mature human platelets is not sufficient to affect platelet function.[14,15] Accordingly, the level of laboratory-measured incomplete response to ASA for the doses currently used in cardiology is rather small.

In the case of clopidogrel, the dose–response curve has all the characteristics of a normal distribution, [12] with higher antiplatelet effect occurring at a loading dose of 600 mg and a maintenance dose of 150 mg a day.[16]

Yet, a clinical problem related to the use of antiplatelet agents is achieving an effective and clinically relevant inhibition of platelet

function with acceptable safety. The high interindividual variability[17] is problematic and might be overcome to some extent, at least in the case of clopidogrel, by increasing loading and maintenance doses. Frequently, the mechanisms underlying variability have been referred to as 'resistance'.

Definitions of 'resistance'

The expected occurrence of incomplete antiplatelet effects of ASA and clopidogrel in some patients started at one point to be defined as aspirin and clopidogrel 'resistance'.[18] Since the beginning, and still now, the main problem with 'resistance' has been the lack of a clear definition, due to the lack of standardized method of platelet function monitoring and of clear and widely accepted cut-off values for platelet function measurements to classify patients dichotomically as either 'responders' or 'non-responders' (resistant).

Following original observations, the term 'resistance' began to be divided into two entities: clinical and laboratory:

- *Clinical 'resistance' to oral antiplatelet drugs* has been defined to be present when a cardiovascular event occurs in a given patient while on antiplatelet drugs;
- *Laboratory 'resistance' to oral antiplatelet drugs* has been defined to be present when *in vitro* platelet reactivity is not properly blocked despite the use of oral antiplatelet drugs.

It should be immediately stressed that such two definitions are not identical: laboratory 'resistance' does not have to end-up with ischaemic events; likewise, having an ischaemic event while on antiplatelet drugs is not necessarily accompanied by laboratory findings of 'resistance', although in a certain—variable—percentage of patients, the two phenomena overlap.[19]

In some recent reviews, it has been proposed to change the name of clinical 'resistance' into 'treatment failure', referring to similar incomplete effects on clinical events occurring after antihypertensive or lipid-lowering therapies, irrespective of a characterization of the aetiology and pathogenesis of such events.[20] The term 'treatment failure' itself can be, however, semantically interpreted in quite different senses. On the one hand, it may refer to a mere assessment of cases occurring while on treatment. It can be imagined, on the other hand, that—in a given patient—a target lipid, blood pressure, and platelet function level (if we could work out such a measure for platelets) is achieved with appropriate therapies, but the patient still develops a cardiovascular ischaemic event. Can we then operatively diagnose such a case as a 'treatment failure', in the sense of a failure of the therapy to prevent those cases that are expected to be preventable by the drug appropriately hitting its own target? The estimates of such treatment failures in the latter strict sense can be easily given with antihypertensive or lipid-lowering therapies, but this is certainly very problematic with antiplatelet treatments.

Therefore, the concepts of 'treatment failure' and of 'clinical resistance' refer to imprecisely defined clinical entities, which cannot, in any case, be easily linked with laboratory measures in the case of antiplatelet treatment.

The opinion of the Working Group

The definitions of clinical resistance or treatment failure related to oral antiplatelet agents are imprecise ways of linking the presence of cardiovascular atherothrombotic events with a possibly inappropriate response to antiplatelet drugs. We therefore propose stopping using such designations in relation to clinical events.

Laboratory resistance

The description of all methods used to measure platelet reactivity in the laboratory is beyond the scope of the current article. Such methods have been described in detail elsewhere.[21–24] We will limit ourselves here to briefly discuss which of the available methods are, in the opinion of the panel, most precisely able to estimate the laboratory effect of ASA and clopidogrel. This is intended to be only a temporary proposition, which can be changed with the possible inclusion of newer methods and the better assessment of current ones.

The methodological problems related with the tests have effectively been described in a recent study where six different tests were compared in a group of patients with stable CHD. All patients were on chronic ASA therapy. Acetylsalicylic acid 'resistance' was diagnosed in percentages varying from 6 to 60% of the subjects, depending on the method used. The results with the various tests hardly correlated with one another.[25] Such estimates highlight the need of using extreme caution when dealing with estimates of *in vitro* platelet reactivity after oral antiplatelet drugs.

Evaluation of the effect of acetylsalicylic acid

First, the proposed 'reference' test to evaluate the *functional* antiplatelet effect of ASA is platelet aggregation induced by arachidonic acid, which is the substrate of the enzyme—COX-1—blocked by ASA. The test can be performed in platelet-rich plasma with the use of optical (transmittance) aggregometry, in whole blood with the use of impedance aggregometry, of thromboelastography and the Platelet Mapping® software, or with the point-of-care VerifyNow® system with disposable cartridges.

Secondly, for all the above-mentioned methods, investigators or device producers have established cut-off values, which could help classifying patients as normal responders or low responders. At present, the majority of such cut-off values relate to optical aggregation induced with arachidonic acid,[26] or impedance aggregometry in whole blood with the same agonist.[27] A low response to ASA is defined as a value above 10–20%[28,29] in optical aggregation or above 0 Ω in impedance whole blood aggregation.[27] It should be stressed that until now there is no proof, by the construction of appropriate receiver operator curves (ROCs), that the given cut-off values are the best in identifying populations with a different cardiovascular outcome. We are actually in the even more worrisome situations that we do not know whether populations separated by such cut-offs are actually different at all in terms of outcomes. The search for such an association link and of possible new cut-off values is ongoing.

Thirdly, the most reliable test for the *biochemical* effect of ASA is the measurement of serum levels of TXB_2 (the stable hydrolytic metabolite of TXA_2) obtained after 1-h whole blood clotting (without anticoagulants) at 37°C. This is an index of the maximum capacity of platelets to produce thromboxane, and is therefore exquisitely sensitive to the biochemical effect of ASA.

As, however, platelet function (including platelet aggregation) depends on the TX pathway to a variable extent, according to the type of activating stimulus and possibly to inter-individual variability, this test cannot be automatically translated into the clinical use to identify low functional (platelet) responses to ASA. Measurements of the urinary output of the hepatic metabolites of TX, such as 11-dehydro-TXB_2, may reflect also extra-platelet production of TX.[30,31] Besides the analytical difficulties, for this test, we also currently do not have clinically relevant cut-off values.

Evaluation of the effect of clopidogrel

First, the proposed *functional* test for clopidogrel is platelet aggregation induced by ADP. It can be performed in platelet-rich plasma with the use of optical transmittance aggregometry, or in whole blood, with the use of impedance aggregometry or of thromboelastography and the Platelet Mapping® software, or with the point-of-care VerifyNow® system.

In one investigation, it appeared that an elevated risk of ischaemic events occurs in patients on clopidogrel showing an aggregation by ADP 20 μM in platelet-rich plasma above 60%, or an aggregation induced by ADP 5 μM above 50%. Such values were worked-out as being above the 75th percentile of aggregation values in a control group.[32] There was apparently no need to estimate the relative changes in aggregation level before and after clopidogrel use in that study.[32]

In other studies, aggregation induced by 20 μM ADP above 50% after a percutaneous coronary intervention (PCI)[2] or residual aggregation induced by 5 μM ADP above 14% (at the end of the recording) before PCI[33] were linked to a heightened risk of recurrent ischaemic events. In patients after drug-eluting stent implantation, aggregation induced by 10 μM ADP $\geq$70% was associated with a higher risk of stent thrombosis in a 6 months follow-up.[34] In diabetic patients on chronic dual antiplatelet treatment, ROC analysis revealed that maximal optical aggregation by 20 μM ADP $>$ 62% was associated with the highest incidence of major adverse cardiac events during a long-term follow-up.[35] One study showed the feasibility of elaborating a score to predict higher residual platelet activity after a loading dose of clopidogrel, which was also associated with a higher incidence of major adverse events.[36] In this prospective study, the cut-off value for aggregation induced by 20 μM ADP was $>$46.9%. A new trial with the use of the VerifyNow® system on the basis of ROC analysis also established device-specific cut-off values in patients after elective PCI with drug-eluting stent implantation.[37] Here, a P2Y12 reaction units (PRU) value $\geq$235 measured after a loading dose of clopidogrel was associated with the higher risk of stent thrombosis or cardiovascular death.[37] Such cut-off values might be used for further verification in large clinical trials.

Secondly, a method to assess the *biochemical* effect of clopidogrel is the flow cytometric analysis of vasodilator-stimulated phosphoprotein (VASP) phosphorylation.[38] This is an expensive and demanding method, but at the same time explores the specific pathway of platelet activation through the $P2Y_{12}$ receptor inhibited by clopidogrel, and therefore is more specific for the effect of clopidogrel than any measurement of platelet aggregation. The cut-off value linked to increased risk of cardiovascular events proposed by some investigators is a 'platelet reactivity index (PRI)' $>$53,[39] a PRI $>$ 48 in another study,[40] and yet a PRI $>$ 50% in a third one.[41] This assay suffers, however, from the same methodological limitations of serum generation of TXB_2 in the assessment of ASA effects: despite reliably assessing the reaching by clopidogrel of its appropriate pharmacological target (the platelet $P2Y_{12}$ receptor), it cannot rule out other confounders in the platelet response to clopidogrel, due to the possible (and actually probable) different dependence of platelet responses on the ADP/$P2Y_{12}$ receptor pathway in different patient populations.

Non-specific methods for measuring platelet reactivity

Other methods for monitoring the effects of antiplatelet agents are less specific. These include the point-of-care PFA-100® device, the Impact cone-and-plate(let)® aggregometer, and optical/impedance aggregometry with the use of agonists other than arachidonic acid and ADP for the assessment of ASA and clopidogrel, respectively (e.g. collagen, thrombin, or other agonists). The results obtained with these devices or systems describe the reactivity of platelet as a whole, and such results can only by far approximation be attributed to the specific pathways blocked by ASA and clopidogrel.[20] However, such tests may be closer to investigating the susceptibility to thrombosis (that the clinician would like to avert) under a certain treatment than some measurements of the biochemical effect of the drug. Some of these tests (all non-automated aggregation tests) are extremely difficult to standardize and subjects to a large variability.

None of the methods so far described (the biochemical tests, measuring the immediate target of the action of a drug, or the functional tests, measuring its more 'distal' effects in reducing platelet function) is therefore ideal, and they are meant to answer different questions. The biochemical tests can assess whether the drug hits its molecular target; the functional tests can assess the extent by which they change platelet function. Both questions are relevant, but neither one is sufficient to guide possible therapeutic decisions. There have been propositions to improve the definition of 'resistance', at least for ASA, based on such considerations. 'Resistance' has been therefore divided into *pharmacodynamic*, *pharmacokinetic*, and *pseudo-resistance*.[42,43] The principles underlying such definitions are in the outcomes of a combination of functional tests (platelet aggregation) and of biochemical tests (TXB_2 concentration in serum or in the supernatant obtained after aggregation). As to clopidogrel, such propositions can be put forward by analogy.

Pharmacodynamic acetylsalicylic acid 'resistance'

Pharmacodynamic ASA 'resistance' may occur because of changes in the target enzyme for ASA, i.e. COX-1. These may include changes in the enzyme structure (due to gene polymorphisms),[44,45] or the transient inaccessibility of the enzyme due to the blockade of the active site by non-steroidal anti-inflammatory agents (e.g. ibuprofen).[46] In such cases, the *in vitro* addition of

ASA to the blood sample would not change significantly the aggregation or TXB_2 levels.[42,43]

Pharmacodynamic clopidogrel 'resistance'

Pharmacodynamic clopidogrel 'resistance' can theoretically occur because of structural changes in the target receptor, the $P2Y_{12}$ receptor (due to gene polymorphisms), although the reports available are not supportive for this.[39,47] It is difficult to perform *in vitro* experiments similar to those with ASA, due to the limited availability of the active clopidogrel metabolite, although another $P2Y_{12}$ receptor antagonist can be considered for this purpose.[48] For the proper diagnosis of this condition, it is also strongly suggested (though difficult to perform) to measure the levels of the active clopidogrel metabolite in the blood to prove its good bioavailability and biotransformation.

Pharmacokinetic acetylsalicylic acid 'resistance'

Here the main reason for a low response to the antiplatelet drug is the limited availability of the active drug at the level of its target. Adding ASA *in vitro* to the blood sample should block or significantly reduce the aggregation as well as TXB_2 concentration.[42,43] Such situations may derive from insufficient dosing or changes in drug absorption (e.g. with the use of enteric-coated formulations, less well absorbed in the intestine). One should here also consider the increased production of new, more active platelets, which cannot be blocked with once daily ASA administration in a relatively small dose.[14]

Pharmacokinetic clopidogrel 'resistance'

Pharmacokinetic clopidogrel 'resistance' can be induced by insufficient dosing, changes in drug absorption, and impairment in the conversion of the pro-drug to the active drug in the liver (e.g. for gene polymorphisms for the enzymes in the chain of pro-drug biotransformation).[47,49] Of note, it has been recently shown that a polymorphism in the gene encoding for the 2C19 isoenzyme is associated with clopidogrel non-responsiveness independent from non-genetic risk factors.[50] An additional reason, as in case of ASA pharmacokinetic 'resistance', might be the (hypothetical) elevated production of newly produced platelets with a higher density of $P2Y_{12}$ receptors.

Apart from the above propositions of systematically classifying the phenomenon of 'resistance', there are other situations that can be classified as 'pseudo-resistance': these include the transient COX-2 expression in new platelets, extra-platelet sources of TX, or the delivery to platelets of substrates (from endothelial cells or monocytes) for TX production bypassing COX-1 blocked by ASA.[51]

It is important to mention at this point that one of the reasons for incomplete platelet blockade with clopidogrel or aspirin can be simply patient's non-compliance.[52] The problem can be as frequent as 17% or more for aspirin[53] and 15% or more for clopidogrel.[54] These percentages are—notably—similar to those reported for aspirin or clopidogrel 'resistance', on the basis of some laboratory estimates, indicating non-compliance as a major issue to be addressed in patients at risk of thrombotic vascular occlusion.

In the opinion of the Working Group, laboratory 'resistance' should be better—and relatively easily—identified as pharmacodynamic 'resistance', where the increased residual platelet reactivity despite the administration of an antiplatelet drug is due to an inadequate blockade of the target enzyme (as to ASA) or receptor (as to clopidogrel).

The opinion of the Working Group—for research purposes only

(1) The term 'laboratory resistance' to oral antiplatelet agents should be reserved to situations when the expected effect from an oral antiplatelet drug cannot be obtained due to changes in the target enzyme or receptor (pharmacodynamic 'resistance'). Such situations can be ascertained with a good approximation *in vitro*.

(2) For the assessment of ASA-specific effects, the proposed test is the use of aggregation induced by arachidonic acid and of TXB_2 concentrations in serum (or in the supernatant after aggregation). For further evaluation, the *in vitro* addition of ASA can be performed before aggregation or the preparation of serum to exclude pharmacokinetic 'resistance'.

(3) For the assessment of clopidogrel-specific effect, the proposed test is aggregation induced with ADP or VASP phosphorylation. For further evaluations, the *in vitro* addition of the active metabolite of the $P2Y_{12}$ receptor antagonist can be performed before such tests to exclude pharmacokinetic 'resistance'.

(4) In the case of abnormal results of non-specific tests, one should only use the term 'elevated platelet reactivity despite treatment'. To detect the reason for this, more specific tests for a given drug should be used.

The extent of the 'resistance' phenomenon

ASA 'resistance' has been reported to occur on the average in 27.1% of patients (95% CI: 21.5–32.6%), according to a recent meta-analysis, but here too with an extremely wide range, from 0 to 57%.[55] As for clopidogrel, this difference can be ascribed to different definitions, different populations studied, different laboratory methods, and different agonist doses within the same laboratory test. For ASA, different prevalence figures in different studies may also be due to different doses, with higher doses reported to overcome 'resistance' in some subjects,[56,57] highlighting that some reasons for this variability can be in the pharmacokinetics of the drug. The above-mentioned frequency of ASA resistance is obtained from different studies using different laboratory protocols. Restricting the analysis only to those studies using aggregation induced with arachidonic acid and/or measurements of serum TX yields an average prevalence of the phenomenon of 6% (95% CI: 0–12%).[55]

The frequency of clopidogrel 'resistance', investigated mainly with the use of optical transmittance aggregation induced by ADP, ranges from 5 to 44%, based on the data available.[58,59] According to a recent meta-analysis, the incomplete inhibition of platelets by clopidogrel in laboratory testing can be seen in about 21% of patients, i.e. one out of five patients undergoing PCI.[60] As for the newer flow cytometric analysis with VASP, the prevalence of 'resistance' is

around 30%.[38] A prevalence of 6% has recently been reported for the combined 'resistance' to aspirin and clopidogrel.[61]

This extremely large variability in the assessment of the prevalence of 'resistance' is a reflection of the variable definitions with the various currently available laboratory tests, the possible existence of pre-analytical and analytical errors, the heterogeneity of different groups studied, as well as differences in research protocols used, drug dosing, and the lack of standardized predefined cut-off values. It has to be noticed that, as for ASA, also in the case of clopidogrel the prevalence of 'resistance' depends strongly on the dose used, showing that true resistance (pharmacodynamic resistance) is rather rare. A lower prevalence of 'resistance' has been found in patients receiving a 600 mg loading dose compared with patients receiving 300 mg,[62] and—for chronic maintenance dose—in patients receiving 150 mg/day compared with 75 mg/day.[63]

The opinion of the Working Group

An exact estimate of the prevalence of 'resistance' to oral antiplatelet drugs is at present impossible. Such impossibility is mainly due to the absence of a univocal definition and of established laboratory methods.

Monitoring of antiplatelet treatments

Every day the practitioner encounters patients with cardiovascular events due to atherothrombosis despite treatment with oral antiplatelet agents. A special category includes rare but severe cases of stent thrombosis, likely more frequent in patients implanted with drug-eluting stents.[64] One has to keep in mind that the currently used antiplatelet drugs do not erase platelet function, and that platelets are not the only cause for those events.[54,65] Methodological problems put the monitoring of antiplatelet effects at a very early stage of standardization for daily clinical practice. This concern is also reflected in the recent European Society of Cardiology Guidelines for non-ST-elevation Acute Coronary Syndromes, which do not recommend the routine use of such monitoring.[66] The American Heart Association/American College of Cardiology went, however, a step forward,[67] asserting that the assessment of clopidogrel antiplatelet activity can be considered in patients in whom possible stent thrombosis might lead to devastating complications. This category comprises patients after unprotected left main stenting, left main bifurcation or stenting of the last patent coronary vessel (Class IIb, Level of Evidence: C). According to such Guidelines, one should consider doubling the chronic clopidogrel dose up to 150 mg/day in patients with $<$50% blockade of platelet aggregation. There is no information, however, about the method of aggregation, type of agonist, and its concentration, and also whether the 50% value quoted above relates to absolute aggregation or relative aggregation compared with the value before drug intake. At the same time, there is no recommendation related to ASA 'resistance'.

Proceeding in situations with incomplete response to antiplatelet drugs

Similarly, there are no recommendations for 'treating' potentially found incomplete response 'discovered' with some way of monitoring. Some investigators have proposed to consider the use of GP IIb/IIIa as an additional treatment during elective angioplasty in such 'resistant' patients;[68,69] others have suggested increasing clopidogrel dose up to 150 mg for chronic use, especially in diabetic patients;[70] one may also consider adding another drug, such as cilostazol, as a third antiplatelet drug.[71,72] It is prudent also to consider the switch to the use of the older thienopyridine available—ticlopidine—in cases of non-responsiveness to clopidogrel. Such an approach can result in achieving pre-defined platelet aggregation level in 83% of primarily clopidogrel 'resistant' patients.[73] New antiplatelet drugs, now under investigation, might also theoretically be of additional value in such situations. Recent clinical data show that the new thienopyridine prasugrel exerts a more potent antiplatelet action than clopidogrel, which can translate into a lower incidence of stent thrombosis, although the risk of severe bleeding is also increased.[74] Prasugrel has so far been tested in a general population with acute coronary syndromes without an individual guidance by platelet function tests. In order to accept the possibility of a selective approach in individual patients, such a proposition should be specifically tested in randomized, prospective trials.

However, a 'greater-than expected platelet reactivity while on treatment' cannot easily be ignored. In certain situations and in certain groups of patients, a high residual platelet activity is connected with a poor prognosis.[75] This has been proved in patients on ASA and clopidogrel, with the use of more or less specific point-of-care methods.[37,76–80] It should also be noted that a high residual platelet activity during treatment with antiplatelet drugs has been consistently shown in diabetic patients,[81] especially when on insulin therapy,[82] obese patients,[83,84] patients with hypercholesterolaemia,[85] and smokers.[86] The same has been reported in patients with acute coronary syndromes.[87] Taking this into account, the most obvious practical strategy would consist in intensively modifying risk factors with a potential influence on platelet function and on the response to antiplatelet drugs. The *in vitro* platelet reactivity is also closely connected with individual compliance. The percentage of non-compliant patients both with regard to ASA and clopidogrel may be as high as 18%.[88] At every contact with the patients, the practitioner should check compliance and remind the patient of its importance, because a most common cause of 'resistance' is patient's 'resistance to take' ASA or clopidogrel.

The European Society of Cardiology recommends, for chronic use, ASA in doses of 75–150 mg and clopidogrel 75 mg/day. This recommendations derives from the Antiplatelet Trialist's Collaboration meta-analysis,[9] where it was shown that such low doses of ASA retain the same efficacy as higher doses with lesser risk of bleeding, and from the CURE trial.[89] Other investigators argue that a lower percentage of ASA-'resistant' patients are in groups where ASA dose is higher.[55] For this reason, some currently ongoing trials (such as CURRENT/OASIS-7) are re-evaluating higher doses of ASA and/or clopidogrel. Such trials are still designed to monitor the antiplatelet effect of ASA and clopidogrel, but without the guidance from an *in vitro* individual evaluation of platelet reactivity. The main combined aim of such trials is to investigate whether increasing the dose of antiplatelet agents would improve the clinical outcome. Some other trials, instead the ASpirin

non-responsiveness and Clopidogrel Endpoint Trial (ASCET), the Gauging Responsiveness with a VerifyNow Assay-Impact on Thrombosis and Safety (GRAVITAS) trial, and the Tailoring Treatment with Tirofiban in Patients Showing Resistance to Aspirin and/or Resistance to Clopidogrel (3T/2R) trial[69] (see http://clinicaltrials.gov), are especially directed to groups of 'resistant' patients.

The ASCET study is a randomized trial where 1000 patients with stable CHD are initially tested with a broad set of platelet function tests while on ASA 160 mg/day, and then, after randomization to continued ASA or clopidogrel 75 mg/day, subsequent evaluations are performed. Patients are then prospectively followed for the evaluation of clinical endpoints during 2 years.[90]

The GRAVITAS study is an ongoing trial randomizing patients 'diagnosed' as clopidogrel 'resistant' according to the VerifyNow© P2Y12 test results after elective coronary PCI with drug-eluting stent implantation into a group on standard maintenance clopidogrel dose or 150 mg daily dose. The primary composite endpoint of the study is cardiac death, non-fatal myocardial infarction, or stent thrombosis at a 6 month follow-up.

The 3T/2R, the results of which were presented during the latest European Society of Cardiology Congress in Munich, randomized 263 patients who were poor responders to aspirin and/or clopidogrel based on VerifyNow© tests results and undergoing elective PCI to receive either high-dose bolus tirofiban or placebo on top of standard aspirin and clopidogrel therapy. The primary endpoint was the occurrence of peri-procedural myocardial infarction, as defined by an increase in Troponin I or T greater than three times the upper limit of normal within 48 h, which was attained in 20.4% of patients treated with tirofiban compared with 35.1% of patients treated with placebo. This resulted in a significant reduction of major adverse cardiovascular events within 30 days in the tirofiban group compared with the placebo group (21.2% vs. 36.6%, respectively; $P = 0.0065$).[69]

Such trials are answering the need at this time to bring the entire issue of 'resistance' to oral antiplatelet drugs to full circle, assessing whether clinical outcomes (myocardial infarction, severe bleeding, death) can be improved as a result of changing therapy as a consequence of the results of platelet function tests. Promising results in this direction, although in small patient groups, have recently been reported.[91]

The opinion of the Working Group

(1) At the present time, there are no clinical data obtained from prospective trials in sufficiently large numbers of patients, showing that the routine or even the occasional determination/monitoring of platelet function while on therapy with antiplatelet drugs and consequent therapeutic decisions leads to any practical clinically relevant advantage. In the absence of such outcome data, and for the vagueness of some current recommendations on the method to be used, any such recommendation, even in the setting of potentially lethal situations, appears premature and de facto impossible to implement in practical terms at the current stage of knowledge.

(2) Whenever the suspicion of 'resistance' is raised by laboratory tests, the immediate practical consequence should be an assessment of compliance to the recommended drugs.

(3) In academic centres with experience in platelet reactivity tests, individual antiplatelet dosing might be implemented in individual cases (e.g. patients with multiple cardiovascular risk factors and recurrent thrombotic events—e.g. stent thrombosis—despite proven compliance to standard antiplatelet drugs doses). Those actions should be undertaken as a research activity, and are currently not based on evidence of efficacy.

Perspectives: authors' point of view

At the current stage, it seems that setting up appropriate cut-off values for platelet reactivity connected with a worse/better clinical outcome is necessary for a classification of patients as 'responders' or 'low-' or 'non-responders' (ROC analysis seems to be the best suited to this regard). Such an assessment of the prognostic implications of results of laboratory tests is preliminary to any practical intervention trial. Such an approach should also define sensitivity and specificity of each laboratory methods with regard to clinical events, which hopefully would lead to the clinical use of the best such test(s) in the future.

Currently ongoing research could also indicate particular groups of patients where a higher residual platelet function is found despite chronic treatment with antiplatelet drugs. Such patients might have a higher potential for profiting from platelet function monitoring or might require an upper titration of the dose even without the setting up of cumbersome platelet function monitoring. Such studies could allow a preliminary assessment of the cost-effectiveness of platelet reactivity tests. Such groups will probably include patients with diabetes ('diabetic' platelets are *per se* more activated), patients after an ischaemic cerebro-vascular accident, patients with peripheral artery disease, and patients undergone coronary bypass surgery or with a history of stent thrombosis. Such patient groups might be candidates, based on the results of the tests, to prospective randomized trials in which the consequences of changing therapeutic regimens based or not on the results of the test(s) will be assessed in terms of relevant 'hard' clinical outcomes (myocardial infarction, severe bleeding, death).

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