Interpretation of clopidogrel resistance

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Clopidogrel resistance has been introduced recently to refer to treatment failure in some individuals received standard clopidogrel therapy [1]. This phenomenon has encountered all who have tested clopidogrel efficacy by platelet aggregometry.

Recently, Dziewierz et al., have discussed the issue of the „inter-individual variability in response to clopidogrel in patients with coronary artery disease" [2]. Their results were in agreement with many published data in the field, which show 10 to 30% prevalence of clopidogrel resistance in the patients undergoing elective coronary artery stenting [3, 4]. Here we would like to emphasis on the metod used and the interpretation of clopidogrel resistance, in patient group reviewed by Dziewierz and associates.

First, it is well known, that clopidogrel metabolites are selective and irreversible antagonist of the ADP receptor P2Y12 [5] and mutations in the P2Y12 gene are associated with a congenital bleeding disorder and any abnormality in the platelet response to ADP resembling that induced by thienopyridines [6]. In this context, ADP seems to be the most appropriate aggregating agent to test the clopidogrel antiplatelet effects. However, ADP activates platelets aggregation through 3 independent receptors. Thus, use of ADP activation via different ADP receptors is debatable, given the fact that the relative contribution of ADP independent receptors in platelet activation was not fully studied to date. Classical pharmacological parameters such as receptor up-regulation and/or changes in receptor sensitivity/threshold for other ADP receptors (e.g. P2Y1) are all theoretically possible when the counter receptor P2Y12 function had been blocked by saturating concentrations of specific antagonists as clopidogrel. Therefore, ADP induced platelet aggregation may not be the most suitable test to measure the individual response to clopidogrel.

The inhibitory effect of ADP acting selectively via P2Y12 receptor decreases PGE1-dependent cell signalling via cAMP-dependent phosphorylation of VASP [7]. Thus measurement of the extent of ADP-induced inhibition of adenylyl cyclase, or VASP phosphorylation will be more appropriate biomarkers to evaluate clopidogrel resistance. Aleil et al. have evaluated the flow cytometric analysis of intraplatelet VASP phosphorylation method recently [8]. They conclude that the phosphorylation of VASP is selectively dependent on the level of activation of platelet P2Y12 receptor and therefore it could be used as a specific test to evaluate the efficacy of clopidogrel treatment and detecion of clopidogrel resistance [8].

Second, so far, no clean definition for clopidogrel resistance is commonly accepted. Studies have shown a dose and time dependent variability in response to clopidogrel treatment as measured by optical platelet aggregometry. In Dziewierz et al. study, clopidogrel resistance were defined as ≤10% reduction in platelets aggregation in response to 20 µmol/L ADP 24 hours after clopidogrel treatment compared with pretreatment values. It is worth to note that (1) Using the same definition and similar patient population, Gurbelet et al. reported clopidogrel resistance in 63% of patients at 2 hours, 31% at 5 days, and 15% at 30 days [9]. However, in most patients, the 30-day inhibitory response from clopidogrel was predicted by the 5-day response [4]. Therefore, 24 hours might not be enough to evaluate clopidogrel resistance. (2) In a study conducted by Muller and asociates, it was found that to 5 µmol/L ADP, 5% were nonresponders and 9% were semiresponders, and to 20 µmol/L ADP, 11% were

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nonresponders and 26% were semiresponders [4]. This study highlights the discrepancy of the results obtained using high and low ADP concentrations in evaluating clopidogrel resistance. However, most of the recent studies combine both low and high ADP concentrations in evaluating clopidogrel resistance.

Third, clopidogrel resistance is measured as a percentage of decreased platelet aggregation response ex vivo to ADP after clopidogrel treatment compared to pretreatment. This relative value is critically dependent on the pre- and posttreatment platelet reactivity. It is known that elective coronary stenting show an increase in platelet reactivity, not all patients exhibited high platelet reactivity [10]. Furthermore, it has been shown that clopidogrel responsiveness after the standard regimen for coronary stenting is dependent on the pretreatment reactivity [3, 11]. Most of the patients with high pretreatment platelet reactivity remainded the most reactive at 24 hours after treatment [3] and despite being more responsive to clopidogrel, patients with moderate reactivity posttreatment [11]. Therefore, evaluating clopidogrel resistance alone might overestimate the risk for stent cardiovascular events in nonresponders with low pretreatment reactivity and underestimate the risk in those responders who remain with high posttreatment platelet reactivity.

In conclusion, clopidogrel resistance could be evaluated more accurately by measuring the phosphorylation of VASP rather than classical measurement of platelet aggregation in response to ADP. Measuring platelet reactivity might be as important as measuring the responsiveness to clopidogrel per se in evaluating the risk of cardiovascular events in patients undergoing coronary artery stenting and/or other interventional procedures.

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Interpretation of clopidogrel resistance. Authors' response

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We are thankful to Dr Almsherqi and his colleagues for their interest in our study and their comments concerning clopidogrel resistance identification and interpretation [1]. However, clarification of several important issues related to this topic are still needed, especially from the clinician's point of view.

First, the aim of our study was to assess usefulness of simple, rapid whole blood platelet function assay (Plateletworks Aggregation Kits, Helena Laboratories, US) in early identification of incidence of clopidogrel resistance [1]. Early identification of non-responders may allow to optimise antiplatelet therapy administered during percutaneous coronary intervention procedures, especially in acute coronary patients, by addition, for example, of intravenous glycoprotein IIb/IIIa inhibitors which can achieve superior platelet inhibition in comparison to clopidogrel alone [2, 3].

The VASP phosporylation state method, mentioned by Dr Almsherqi, is one of many platelet function tests which can be applied to cardiovascular disease as a way to predict clinical outcomes and to monitor effects of antiplatelet drugs [4-6]. We agree that it could be used as a specific test to evaluate the efficacy of clopidogrel treatment. However, this method has several limitations (time-consuming sample preparation, expensiveness, requirement of flow cytometer and experienced operator) which reduces its usefulness in everyday clinical practice, especially in diagnosis and treatment of acute coronary patients [4]. Reports about prediction of clinical outcomes using this method are also limited [4, 5].

The Plateletworks assay, unlike VASP phosporylation state, is a simple, point-of-care, time-saving method based on a standard automated cell counter [4, 7]. Therefore it can be used in hospitals and catheterization laboratories offering 24/7 service to monitor the effect of platelet inhibitors during percutaneous coronary interventions, especially in acute coronary patients. Others have shown that results obtained by this method correlate well with the results obtained by the use of optical aggregometry and other whole blood platelet function assays [7, 8]. The Plateletworks Aggregation Kits method is also able to determine combined antiplatelet treatment effects (including glycoprotein IIb/IIIa antagonists) in total [4]. Therefore, it may be important for predicting long-term clinical outcome [9].

We agree that, so far, there is not a standardised definition of clopidogrel resistance, nor clinically confirmed platelet aggregation inhibition cut-off value to identify non-responders to clopidogrel [10]. In our study we used the most popular definition proposed by Gurbel et al. [11]. On the other hand, Serebruany et al. has recently shown, in a cohort of 544 individuals, a normal, bell-shaped distribution of response to clopidogrel [12]. In this paper platelet response to clopidogrel was defined as follows: hypo-responders (two standard deviations below the mean), hyper-responders (two standard deviations above the mean); and the remaining subjects were defined as normal responders [12]. This definition seems to be more physiologic than that based on arbitrary platelet aggregation inhibition cut-off value, because the optimal level of platelet aggregation inhibition to prevent cardiovascular events may vary in different patients' subsets.

Our study focused on early effects of clopidogrel treatment (within 24 hours), which may be crucial for predicting cardiovascular events during follow-up [1]. We fully agree that 24 hours may not be enough for definitive confirmation of non-response to clopidogrel [13]. Regardless, our paper supports that early

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identification (6 hours from the first dose of clopidogrel) of non-responders is possible and clinically feasible. The response to therapy measured at 6 hours correlated well with that assessed at 24 hours [1]. Others have documented that platelet aggregation inhibition examined at 24 hours highly correlates with that measured 5 or 30 days from the initiation of clopidogrel treatment [13].

It is well known that baseline platelet activity before administration of a loading dose of clopidogrel seems to be an important factor determining response to treatment. Also, in many studies, an increase in platelet activity after coronary stenting was observed [11]. To avoid influence of coronary stenting on platelet inhibition measurements and achieved results, in our study all blood samples were taken before percutaneous coronary intervention [1].

In conclusion, results of many studies demonstrate a wide range of responsiveness to clopidogrel in patients with cardiovascular diseases [1, 5, 11, 12, 14, 15]. To date, there are only a few small trials that have explored the clinical relevance of an inadequate response to clopidogrel [5, 14, 15]. Large scale clinical trials to correlate platelet function measurements with clinical outcomes are still needed. If these measurements will correspond to the risk of cardiovascular complications during follow-up, rather simple methods of platelet function testing may be useful in every day clinical practice for the optimisation of the antiplatelet therapy in the future.

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