Prevalence of clopidogrel resistance evaluated by the VASP method

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Abstract

BACKGROUND: Despite oral antiplatelet treatment a certain number of patients suffer adverse events while on therapy. Whether patients demonstrating low platelet inhibition in vitro are at increased risk for adverse events is unknown. The prevalence of clopidogrel resistance ranges from 4-60% in the literature and a standardized cut off value for determining resistance, protocol for treatment and testing is lacking. Characteristics of the clopidogrel resistant patient are unknown.

OBJECTIVES: The aim of the study was to determine a comparable, clinically applicable reference cut-off value for clopidogrel resistance with the VASP method in patients with coronary artery disease (CAD). Furthermore we sought to explore the prevalence of resistance in the same population while on clopidogrel treatment, and within defined patient sub-groups.

METHODS: Vasodilator-stimulated phosphoprotein (VASP) analyses, a standardized flow cytometric assay which yields a VASP platelet reactivity index (PRI) value indicating residual platelet activity, were performed on whole blood samples from 158 patients with angiographically documented CAD on clopidogrel treatment. The cut-off value for resistance was defined by the 5%-percentile in a control group of patients with CAD (n=105) being on aspirin.

RESULTS: The cut-off value for clopidogrel resistance was determined to be VASP PRI $\geq$ 55. 29.7% of the patients had a VASP PRI $\geq$55 while on clopidogrel treatment. We did not find any correlation between clopidogrel resistance and age (r=0.005, p=0.952). There were also no associations with gender (p=0.596), smoking habit (p=0.523), hypertension (p=0.445) or diabetes (0.498).

CONCLUSIONS: VASP analysis could be useful for monitoring response to treatment and tailoring antithrombotic drug regimens for CAD patients. This would be of great importance if future prospective clinical studies show that clopidogrel resistant patients are at higher risk for adverse events than patients with an acceptable platelet inhibition with clopidogrel.

Contents

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Background

The antithrombotic anti-platelet agents aspirin and clopidogrel are widely used when treating patients with atherotrombotic disease in cardiovascular or other vascular beds. Administrated alone or in combination, both have proven efficacy when it comes to reducing the risk of adverse events, such as myocardial infarction, stroke or vascular death (1). The results of the CAPRIE study showed clopidogrel to be superior to aspirin (p=0.043) in reducing the risk of ischaemic stroke, myocardial infarction and vascular death (2) and in the CURE study the benefit of adding clopidogrel to aspirin treatment was demonstrated (dual therapy was more effective than aspirin and placebo) (3). The ongoing ASpirin- and Clopidogrel non-responsiveness clinical Endpoint Trial (ASCET) will explore whether clopidogrel can improve the clinical outcome during a two year follow-up in patients with coronary artery disease (CAD) with low initial response to aspirin in laboratory tests (4).

The thienopyridine clopidogrel acts by blocking adenosine diphosphate (ADP)-dependent aggregation of platelets. ADP is obligate for the release of more ADP from platelet granula which reinforces the aggregation induced by ADP itself or its agonists. Furthermore ADP mediates down-regulation of the adenylyl cyclase in the platelet, thus decreasing cyclic adenosine monophosphate (cAMP) production and facilitating platelet activation and aggregation. Clopidogrel is a pro-drug, converted to its active metabolite, a thiol derivate, through oxidation and hydrolysis by hepatic CYP450 isoenzymes. The active form binds selectively and irreversibly to one of the two ADP-receptors of the platelet; P2Y12 and P2Y1. P2Y12 is a 7-transmembrane receptor coupled to a G_i-protein that triggers multiple cellular pathways when stimulated (5). Dephosphorylation of the intracellular vasodilator-stimulated phosphoprotein (VASP) is dependent on P2Y12-receptor stimulation. Inhibition of P2Y12 by clopidogrel induce phosphorylation of VASP by c-AMP-dependent protein kinases. A low level of VASP-phosphorylation therefore reflects receptor activation, while high levels of phosphorylated VASP reflect P2Y12 inhibition (6). This can therefore be used to measure the degree of platelet inhibition.

Despite oral antiplatelet treatment a certain number of patients suffer myocardial infarction, stroke or vascular death while on therapy, formerly named “clinical resistance” or “treatment failure”. A likely hypothesis is that incomplete platelet inhibition is the main cause. Despite the use of anti-platelet agents, laboratory methods have showed that some patients fail to achieve the expected level of platelet inhibition and demonstrate a high platelet residual activity, i.e. “laboratory resistance”. In vitro analysis have revealed that patients on clopidogrel demonstrates a significantly lower platelet activity than volunteers and patients not receiving clopidogrel (p<0.0001)(6). However the wider range of values and a larger standard deviation in this group demonstrates a great interindividual variability in the response to clopidogrel, which otherwise follows a normal bell-shaped distribution (7,3). We will use the term “resistance” as recommended by the European Society of Cardiology Working Group on Thrombosis, describing a state where in vitro platelet reactivity is not

adequately blocked by oral antiplatelet agents (1). Some studies have been able to show a link between laboratory findings and clinical outcome in patients, and in recent small, clinical studies laboratory findings of clopidogrel resistance seem promising as predictors of clinical outcome (1,8, 9).

A number of mechanisms have been proposed to explain the great interindividual variability in the response to clopidogrel. These include patient non-compliance and failure to prescribe the right dosage, poor absorption of the drug, drug-drug interactions (demonstrated for some statins and clopidogrel), differences in metabolic activity and genetic polymorphisms of the CYP system, platelet variation (life span, ADP volume released, ADP-receptor (P2Y1 and P2Y12) up-and down-regulation) and platelet activation by alternative pathways (10,11).

Measuring ADP-induced maximum platelet aggragtion by light tranmittance aggregometry (LTA), Verify Now and flow-cytometric-based VASP assay are some of the methods that have been applied to detect clopidogrel resistance. Aleil et al showed that flow cytometric VASP phosphorylation state is highly correlated with specific inhibition of P2Y12 - the target receptor for clopidogrel (r=0,72 and p<0,0001) (6). Further advantages of the VASP assay, is that it requires a low sample volume, it can be used with whole blood and it is stable for about 72 h (12). From flow-cytometric analysis and fluorescence measurements of blood samples incubated with natural agonists and antagonists of platelet aggregation and activation (PGE1 and ADP), a platelet reactivity index (PRI) can be calculated, expressed as a percentage value (100% equals full reactivity, i.e. no inhibition of platelets).

In the beforementioned study, Aleil et al found, as expected, that patients receiving clopidogrel had significantly lower PRI than healthy blood donors (volunteers) and patients not on clopidogrel. However, approximately 33% of the patients receiving clopidogrel had a PRI equivalent to that of patients not under clopidogrel treatment, i.e. as if they were not on treatment (6).

Clinical studies exploring resistance to clopidogrel are now numerous, but with the prevalence of resistance ranging from 4-60% (14).

The aim of the present study was to determine a comparable and clinically applicable reference cut-off value for clopidogrel resistance with the VASP method in patients with stable CAD from the ASCET-population (4). Furthermore we sought to explore the prevalence of this phenomenon in the same population while on clopidogrel treatment, and within defined patient sub-groups.

Methods

Study population

The patients included in this project were all participating in the ASCET study which was mainly aimed at investigating the influence of non-responsiveness to aspirin treatment on clinical events. They were 18-80 years of age, of either gender and had angiographically documented CAD being treated with aspirin 160 mg/d for at least one week before inclusion, treated with angioplasty/stent implantation (percutaneous coronary intervention (PCI)) or not. The exclusion criteria were indication for warfarin treatment, contraindications to aspirin or clopidogrel, malignancy that might interfere with life expectancy, psychiatric disease, mental retardation, dementia, drug abuse, alcoholism or conditions thought to reduce compliance. The patients were randomized to either continued aspirin treatment or to clopidogrel 75 mg/d (tablets of Plavix® (Sanofi Winthrop Industries, Ambarese, France and Bristol-Myers Squibb SNC, Paris, France)). The medications were covered by the Act of National Insurance Administration. The study was approved by the Regional Ethics Committee and all patients have given written informed consent to participate.
The present investigation was performed in 158 randomly selected patients one month after being randomized to treatment with clopidogrel. In addition we included 105 patients on aspirin for determination of the cut-off level for the VASP method.

Blood samples

Blood samples for testing platelet reactivity were drawn in fasting condition in the morning before administration of any medication, i.e. about 24 hours after the last dose of clopidogrel. Blood was collected in vacutainer tubes containing citrate (0.129M in dilution 1:10). Samples were kept at room temperature and analyzed within 48 hours.

Laboratory method: VASP phosphorylation

The effect of clopidogrel on platelet function was evaluated in vitro with the analysis of VASP. VASP is an intracellular protein in platelets, which is dephosphorylated in the normal state. Phosphorylation of VASP is regulated by the cAMP cascade. PGE1 activates this cascade, while the cascade is inhibited by ADP via the P2Y12 receptor (Fig. 1).

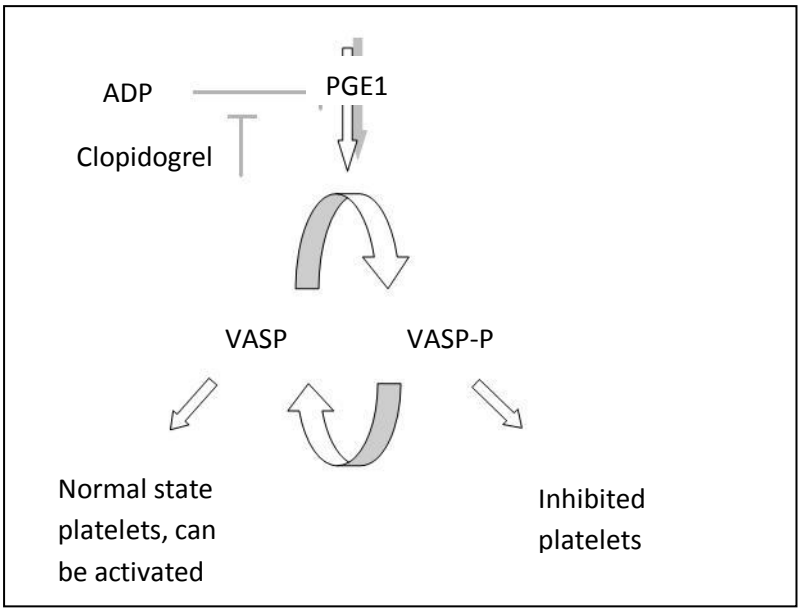

Figure 1: Phosphorylation state of VASP indicates state of platelet activation. PGE1 inhibits platelets, while ADP inhibits the inhibitor. The cAMP cascade is not shown.

To determine the VASP phosphorylation state of whole blood, we used a standardized flow cytometric assay [PLT VASP/P2Y12;Biocytex, Marseille, France]. Citrated blood samples were incubated with PGE1(10 µm) alone or with PGE1 and ADP simultaneously (10 µm) for 10 minutes. Samples were then fixed and the cells permeabilized. Immunolabelling was done using indirect no wash immunofluorescence primary anti VASP-P mouse monoclonal antibody, followed by a secondary fluorescein and polyclonal antibody anti mouse IgG-FITC. Platelet counter-staining reagent-PE (anti CD61-PE) was also used. The duration of the preparation of samples did not exceed 30 minutes, as recommended by the supplier. Analyses were performed on a FACSCalibur flow cytometer (Becton Dickinson, USA) and platelet populations were identified using forward and side scatter.

A platelet reactivity index (PRI) was calculated using corrected (by negative control) mean fluorenscence intensity of samples incubated with PGE1 or PGE1 and ADP according to this calculation:

$$\mathbf{PRI = [(MFI_{C\ PGE1} - MFI_{C\ (PGE1+ADP)} / MFI_{C\ PGE1}] \times 100}$$

Statistical analysis

Continuous variables are expressed as mean ± standard deviation (SD) and categorical variables are presented as frequencies and percentages.

Comparisons between groups were performed by independent-samples t-tests or Mann-Whitney test for continuous variables and chi-square exact test for categorical variables. Coefficient of correlation was calculated by Pearson. $P<0.05$ was considered statistically significant. Statistical analysis was performed using SPSS (v16.0, SPSS Inc).

Results

The demographic and clinical characteristics of the total ASCET population are given in Table 1.

Table 1: Characteristics of the CAD population (n=1001). Values are mean (SD) or number (proportions) if not otherwise stated. SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: body mass index; ACE: angiotensin converting enzyme.

Men/Women (%)	783/218 (78/22)
Diabetes Mellitus n (%)	200 (20)
Myocardial infarction n (%)	436 (44)
Hypertension n (%)	553 (56)
SBP (mmHg)	139.4 (19.3)
DBP (mmHg)	82.1 (9.7)
Current smokers n (%)	204 (20.4)
BMI (kg/m^2)	27.9 (11.5)
Total cholesterol (mmol/l)	4.6 (1.0)
HDL cholesterol (mmol/l)	1.3 (0.4)
LDL cholesterol (mmol/l)	2.5 (0.8)
Triglycerides (mmol/l)	1.6 (1.1)
Fasting glucose (mmol/l)	6.0 (1.9)
HbA1c (%)	6.0 (0.9)
Medication %	
Statins	98
Aspirin	100
β-Blockers	76
Nitrates	22
ACE inhibitors	26

In our sub-study population 124 patients were men (78%) and 34 (22%) women. The number of hypertensives was 94 (59.4%), 27 (17%) were diabetics and 24 (15%) current smokers. With these numbers we find this sub-population to be representative for the whole ASCET population.

Determination of cut-off value

To determine the cut off-value for clopidogrel resistance in our study population we used a control group of 105 CAD patients also from the ASCET population. The control group had received aspirin 160 mg/d for at least one week when their blood was drawn to obtain VASP PRI. Aspirin is a COX-inhibitor, inhibiting platelet activation by another route than clopidogrel does. Aspirin is not known to have any interaction with the P2Y12-receptor, so we consider the VASP PRI obtained in this group to be similar to "normal" VASP PRI values in CAD patients.

VASP PRI in the control group (n=105) (Fig. 2) showed a somewhat skew and broad distribution with a mean of 82.6 +/- 12.4. The minimum was 18.0 and the maximum 96.0. The 5% percentile was 54.8, and ≥ 55 was chosen as the cut-off value for clopidogrel resistance in our study population.

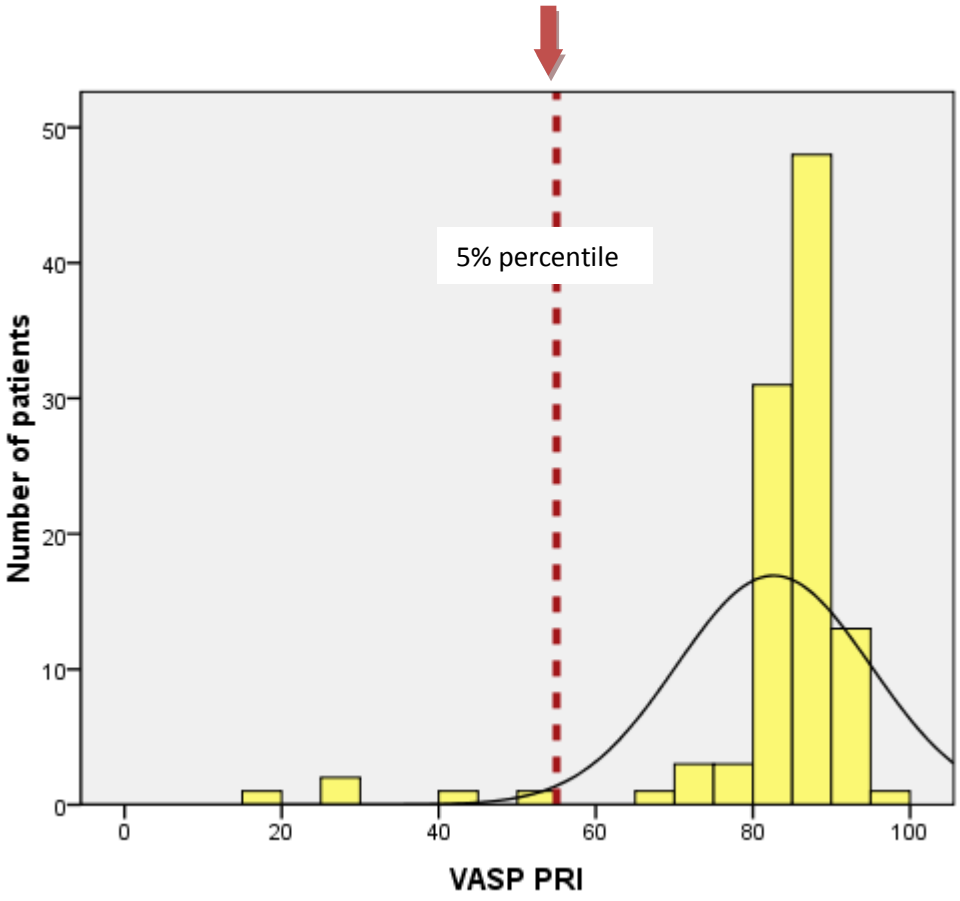

Figure 2: VASP PRI distribution (82.6 +/- 12.4) of a group of 105 CAD patients not receiving P2Y12 antagonist.

Prevalence of resistant patients

The distribution of VASP PRI in the patients on clopidogrel treatment (n=158) gave a mean VASP PRI of 42.0, compared to 82.6 in the control group (p<0.001 (t-test); p<0.001 (Mann-Whitney test). The SD and range were also wider (21.0 vs 12.4) in this group, indicating the expected large interindividual variation in response to clopidogrel (Fig.3).

The VASP PRI in the study group is almost normally distributed, more so than in the control group. Such a distribution permitted the further use of parametric statistical methods in the study when performing analyses on the clopidogrel-treated group alone.

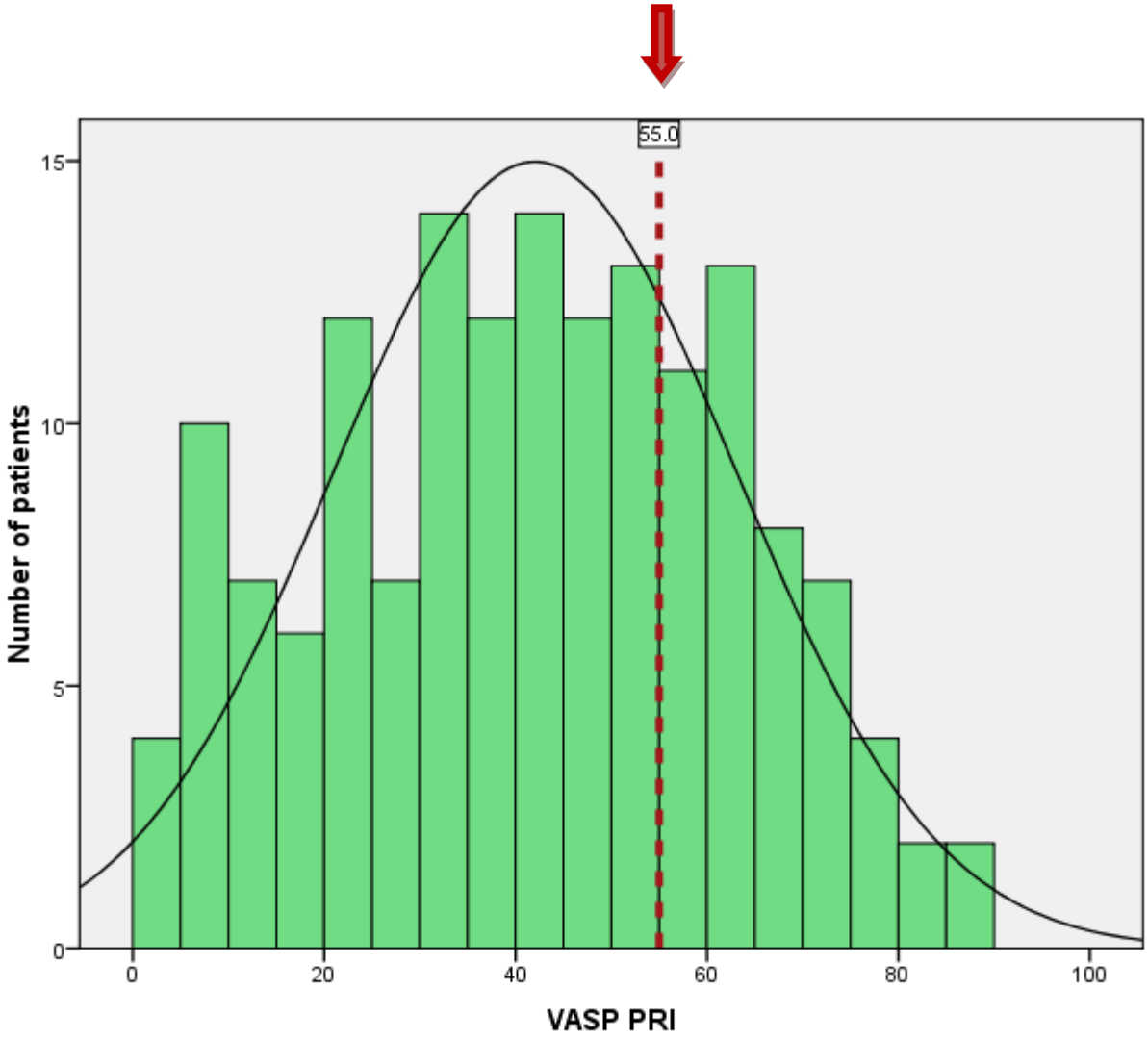

Figure 3: VASP PRI distribution (42.0± 21.0) in patients (n=158) receiving 75 mg Plavix® daily. As also denoted in other literature, the response to clopidogrel is normally distributed and bellshaped (7,3). Patients with a VASP PRI ≥ 55 are to be characterized as resistant to clopidogrel in our study.

Using the defined cut-off value of VASP PRI ≥ 55 for clopidogrel resistance, 29.7% of the patients (47/158) were classified as resistant. (Table 2).

RESPGR

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	0	47	29.7	29.7	29.7
	1	111	70.3	70.3	100.0
	Total	158	100.0	100.0	

Table 2: Frequency of resistant patients. Patients with PRI VASP ≥ 55 in group "0" (non-responder).

VASP-PRI levels within defined sub-groups of patients

VASP PRI levels showed no significant correlation with age ((r=0.005; *p*=0.952) Figure 4). There were also no difference in VASP PRI when comparing the 10% youngest (age ≤51 yrs) and the 10% oldest of patients (age> 76 yrs)(44±27 vs. 47±17;*p*=0.670). When divided into quartiles, comparing the 25% youngest and oldest, the *p* value was 0.925.

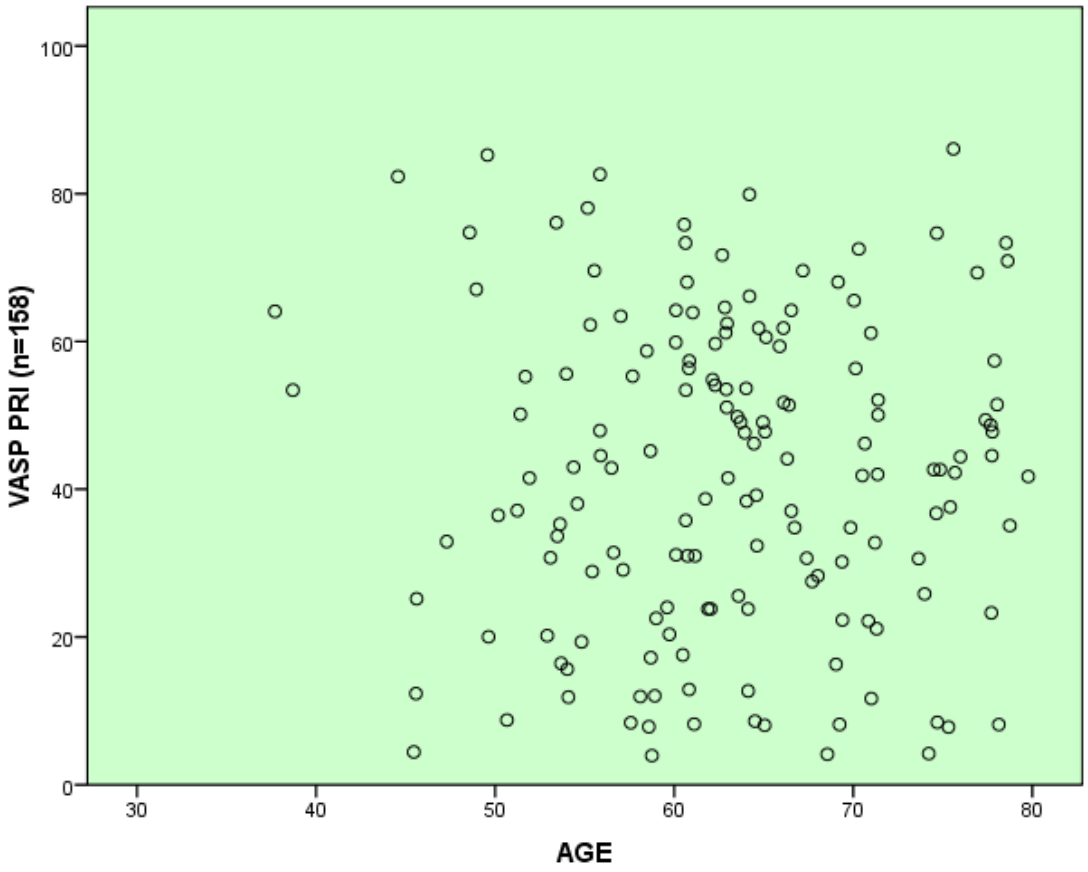

Figure 4: Correlation between VASP PRI and age.

There was no statistically significant difference in VASP PRI levels between men and women (43±22 vs 36±18; *p*=0.067). Nor were there any differences between groups of smokers and non-smokers (42±24 vs. 42±21; *p*=0.957) (Table 3a and b), hypertensive versus non-hypertensive patients (43±20vs.40±22; p=0.421) or diabetic versus non-diabetic patients (42±22 vs. 42±21; p=0.907).

Table 3a and 3b: Tables showing independent samples t-test for comparing means of PRI VASP between smokers and non-smokers, as an example of calculation.

a)

Group Statistics

	SMOKE1	N	Mean	Std. Deviation	Std. Error Mean
VASPPRI2	0	134	42.0653	20.65009	1.78390
	1	24	41.8129	23.51971	4.80094

b)

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
VASPPRI2	Equal variances assumed	.324	.570	.054	156	**.957**	.25238	4.67634	-8.98473	9.48950
	Equal variances not assumed			.049	29.692	.961	.25238	5.12165	-10.21199	10.71675

Clopidogrel resistance according to the defined cut-off value in subgroups of patients

The number of resistant patients did not differ significantly between any of the groups, shown in Tables 4 and 5, and Figure 4.

Table 4: Number of patients being resistant/responders in subgroups of patients

	Resistant patients	Responders	*p*
Male Female	42 5	82 29	0.596
Smokers Non-smokers	7 40	17 94	0.523
Diabetic Non-diabetic	9 38	18 93	0.498
Hypertensive Non-hypertensive	28 19	66 45	0.445

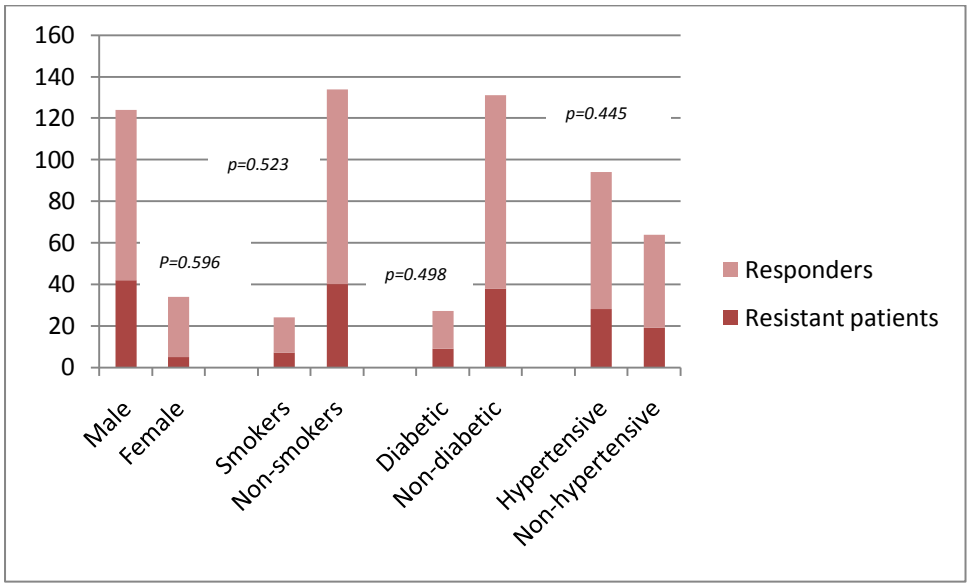

Figure 5: Number of clopidogel resistant patients within different patient groups.

Table 5: Chi-square test to calculate the equality of number of resistant (population proportions) in diabetic/non-diabetic patients, as an example of statistical calculation. Patients are grouped as either responders or clopidogrel resistant within their diabetic/non-diabetic group.

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.474E2	148	**.498**
Likelihood Ratio	136.185	148	.748
Linear-by-Linear Association	.014	1	.906
N of Valid Cases	158		

Discussion

In this study we defined resistance to clopidogrel according to the VASP PRI range in a control group of patients with angiographically documented CAD not receiving clopidogrel. The 5% percentile of this group was VASP PRI 55. Of the patients in the study population 29.7% had a VASP PRI equivalent or superior to this, i.e. were resistant.
This is in line with the results from Aleil et al, who also explored clopidogrel effect by VASP analyses and found that 33% of their patients with cardiovascular disease on clopidogrel treatment had a PRI equivalent to values in patients not on clopidogrel treatment (6).

The prevalence of clopidogrel resistance ranges from 4-60% (14) in different studies. An explanation for this wide range could be the many differences in study protocols applied. For example the dosage of clopidogrel varies (there is controversy in the literature on what is the most efficient dosage). There are also different methods of measurement, differences in patient populations, relatively small patient populations in the studies overall, different time for platelet evaluation in relation to time of administration of drug and lastly there is no univocal definition on how resistance should be defined, in terms of either a certain numeric cut-off value, a cut-off value defined for the population studied based on platelet in-vitro analysis or others.

In our study, VASP PRI levels did not show any associations with age, sex, smoking, hypertension or diabetes. We did not find that the frequency of clopidogrel resistance was significantly higher within any of these subgroups of patients.

Angiolillo et al reported, contrary to what we found, the there were a higher number of clopidogrel resistant patients within a group of diabetic subjects compared to non-diabetic individuals ($p=0.04$). Overall ADP-induced platelet aggregation and platelet activation was also higher in diabetic than in non-diabetic patient in their study (15). In their study resistance was defined as an absolute reduction of <10 in platelet aggregation with ADP 24 h after 300 mg clopidogrel administration compared with baseline values. Patients in this study did also receive aspirin, thus the intervention group had dual therapy with clopidogrel.

There are currently no results available from large clinical prospective randomized trials studying whether clopidogrel resistant patients have a higher rate of adverse events or increased risk of death compared to clopidogrel responders. The data available are mainly observations of patients with different acute coronary syndromes (ACS).

Some of the first to explore this, Matetzky et al, found that in their patient group of STEMI-patients undergoing PCI, 40% of patients exhibiting the highest quartile of ADP-induced aggregation while on clopidogrel treatment, experienced a recurrent cardiovascular event within six months. In the two quartiles with the lowest aggregation values none of the patients experienced such events (16).

Frere et al followed patients having undergone PCI for NSTE ACS (non-ST elevating myocardial infarction acute coronary syndrome) for one month. They found that VASP PRI was significantly lower in patients who did not experience cardiovascular events, than in those who did. The cut off value for detecting patients at higher risk for events was 53 in their material, determined by ROC curve analyses. This yielded VASP PRI a negative predictive value of 99%. (9). A negative predictive value of 100% for VASP PRI was found for excluding major adverse cardiovascular event (MACE) after PCI in patients with NSTE ACS, stable angina pectoris and/or silent ischemia by Bonello et al in 2007 (13). Their ROC analysis showed an optimal cut-off of VASP PRI 50. These cut-off values, based on patient observations, fit well with our statistically defined cut-off (VASP PRI $\geq$55) based on our control group of CAD patients on aspirin.

Clinical prospective randomized controlled trials are needed to determine if clopidogrel resistant patients have a higher rate of adverse events or increased risk of death compared to clopidogrel responders.

It has been suggested that the risk of bleeding and protection from MACE is not correlated with the P2Y12-antagonist used, but rather the degree of platelet inhibition achieved. Should this be true, a VASP PRI cut-off value should be valid for all thienopyridines (5). Thus, despite new thienopridines on the horizon, characterized by faster onset of action and more consistent inhibition, research on clopidogrel, which still maintains the position as the most widely used and safest drug, is not in vain. The importance of agreeing on a study protocol for obtaining comparable results when discussing prevalence of resistance to thienopyridines in different patient populations cannot be emphazised strongly enough.

Our data showed that clopidogrel response varies substancially interindividually, as described elsewhere (7). The mechanisms for clopidogrel resistance is not fully defined and known, but several hypotheses have been presented.

Patient non-compliance should always be thought of. In the case of clopidogrel, inappropriate timing for measuring effect or inappropriate dosing seems more likely as a cause of resistance. As mentioned, there is controversy regarding the optimal loading and maintenance dose of clopidogrel. Aleil et al demonstrated that resistance to clopidogrel is dose-related. In their study of 153 elective PCI-patients the proportion of low responders (VASP PRI≥69, on basis of their previous study (6)) was significantly lower in patients randomized to clopidogrel 150 mg/day than in those randomized to clopidogrel 75 mg/day (8.6% vs. 33.7%; p = 0.0004). In the clopidogrel 75 mg/day group, 64.5% of low responders became responders after switching to clopidogrel 150 mg/day for 2 weeks (17).

In 2008 Bonello et al (8) evaluated the clinical impact of adjusting the loading dose of clopidogrel according to the VASP index in patients with clopidogrel resistance undergoing PCI. Clopidogrel resistance was defined as a VASP >50% after a 600-mg loading dose. Patients with clopidogrel resistance undergoing coronary stenting were randomized to a control group or to a VASP-guided group, in which patients received additional doses of clopidogrel to decrease the VASP PRI below 50. Dose adjustment was efficient in 86% of patients and VASP index was significantly decreased ($p < 0.001$). The rate of major adverse cardiac events was significantly lower in the VASP-guided group ($p = 0.007$). In 2009 they performed a similar study on a larger patient population, with similar results (18).

Further mechanisms for resistance encompasses reduced bioavailability of clopidogrel, either because of poor absorption from the intestine (19), decreased or initially low hepatic conversion to the active metabolite or drug-drug interaction at the enzymatic level. The metabolism of clopidogrel is dependent on the hepatic cytochrome 450 isoenzymes (CYP3A4, CYP2C19, CYP1A2, CYP2B6 and CYP2C9)(20).

Wide interindividual variability in CYP3A4 activity has been demonstrated in both patients and healthy volunteers. Percent platelet aggregation in patients after clopidogrel administration correlated inversely with CYP3A4 activity (r=-0.6, p=0.003). They also noted improvement of platelet inhibition in subjects initially resistant to clopidogrel with coadministration of rifampicin (inducer of CYP3A4) (19).

CYP3A4 enzymes metabolize as much as one half of drugs prescribed (21). Especially statins have been investigated regarding an eventual drug-drug inhibitory interaction with clopidogrel, but without univocal results. In the ASCET study 98% of patients received statins during the trial. Statins, at least alone, seems not to cause resistance. On the other hand St.Johns wort, a herbal supplementation, has been noted to convert clopidogrel non-responders to responders, by significant induction of CYP3A4 enzymatic activity (21).

As for CYP2C19, at least 25 different single nucleotide polymorphisms in its coding gene have been identified. A significant reduction in serum concentration of the active metabolite of clopidogrel and reduced platelet aggregation are associated with the most common of these polymorphisms, the CYP2C19*2 in exon 5. This polymorphism has therefore been called the “loss-of-function” allele (20, 22). Another study on a subpopulation of the ASCET population found that 29% of patients were carriers of the CYP2C19*2 polymorphism. In this subpopulation, as in this study, 29% of patients were clopidogrel resistant when analyzed with VASP. The frequency of clopidogrel resistance in patients with the polymorphism was 46% compared to 22% in wild-type patients (p=0.003). A higher prevalence of resistance was found in patients with prior myocardial infarction (p=0.001) and interestingly also in patients with a BMI above median (27kg/m2) (p=0.015) (23). There is otherwise controversy in the literature on the impact of BMI on clopidogrel response.

When looking for other mechanisms possibly contributing to resistance, it`s important to keep in mind the platelets themselves. An increased platelet turnover, as seen after surgery, trauma and during infection and inflammation, for example, or diseases interfering with the life span of platelets, might have an impact on the effect of clopidogrel in the organism. The amount of ADP released by a platelet when activated might also play a role. Lastly ADP-receptor (P2Y1 and P2Y12) up-and down-regulation and platelet activation by alternate pathways (10, 11) should be considered. Receptor polymorphism has been demonstrated for the P2Y12 receptor, although the effect or lack thereof on modulating platelet response to clopidogrel is controversial (21). If platelet activation in a patient is for a large part mediated by other agonists than ADP and other routes than P2Y12, unsatisfactory inhibitory effect of clopidogrel would be likely.

In the VASP-guided group of Bonello et al`s 2008 study (8), 14.4% of patients maintained a PRI>50, even after having received as much as four doses, 2400 mg, of clopidogrel. In a recent review, insufficient metabolite generation as the primary explanation for nonresponsiveness to clopidogrel, rather than genetic polymorphisms of platelet receptors or intracellular signaling mechanisms was discussed (14). In patients who remain resistant and demonstrate high platelet activity despite high doses of clopidogrel, on the other hand, they suggest that the cause is genetic polymorphism (14). There might thus not be only one mechanism for clopidogrel resistance.

In summary

The VASP method has so far been used only for research purposes, but in vitro evaluation of platelet inhibition to identify non-responders or resistant patients on antiplatelet agents might very well be the future of clinical practice. The ongoing ASCET study, the first large prospective study on-aspirin non-responsiveness, will show whether aspririn non-responders according to laboratory testing, are at higher risk for adverse events than patients with acceptable-response to aspirin. Similar prospective studies on clopidogrel are needed in the future. Should such results indicate that resistant patients have a higher rate of adverse events or increased risk of death compared to clopidogrel-respondant patients, VASP PRI evaluation could be used by the physician to determine whether a patient should receive clopidogrel or if another antiplatelet drug would give more optimal platelet inhibition.

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