CLOPIDOGREL NON-RESPONSIVENESS IN PATIENTS WITH CORONARY HEART DISEASE

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Summary

Background

Clopidogrel is an antiplatelet drug, blocking the ADP-pathway of platelet activation. Currently there is no universally accepted definition of *clopidogrel non- responsiveness*. The difference between pre-treatment and posttreatment ADP- induced platelet aggregation has been used as an estimate of clopidogrel non- responsiveness.

The aim of the present study was to define a cut- off point for clopidogrel non- responsiveness in a study population of patients with stable coronary heart disease (CHD), and possibly give an estimation of the prevalence of non- responders in patients with stable CHD.

Methods

The study population consists of 227 consecutively enrolled patients from the ASCET-study, all treated with ASA, of which 91 were later randomized to clopidogrel. Analysis on venous blood samples were performed using VerifyNow $P2Y_{12}$ assay, which measures ADP-induced platelet aggregation. We performed descriptive statistics on the outcome.

Results

The 5% percentile of Platelet Reaction Units (PRU) at baseline was close to 170, and we used this as our cut- off- point for non- responsiveness. The equivalent 95% percentile value in % inhibition was 24%. After one month on clopidogrel treatment, we calculated a prevalence of non- responsiveness to clopidogrel of 35% and 28% for PRU and % inhibition respectively.

Conclusions

We found a frequency of clopidogrel non- responsiveness (28-35%) that corresponds with current literature (5-44%). As ex vivo platelet function tests not necessarily give an accurate measurement of in vivo platelet function, clinical non- responsiveness needs to be explored in larger prospective studies.

Introduction/ background

Platelet aggregation plays a major role in the pathogenesis of cardiovascular disease. Before clopidogrel was introduced, acetylsalisylic acid (ASA) was the generally used antiplatelet drug for long term treatment. ASA is an irreversible cyclooxygenase 1- inhibitor.
In general clopidogrel is an antiplatelet drug, blocking the ADP-pathway of platelet activation. Clopidogrel (Plavix®) is used for prevention of atherotrombotic events in patients with acute coronary syndrome (unstable angina pectoris or acute myocardial infarction) and in patients undergoing Percutaneous Coronary Intervention (PCI) with stenting of coronary-, carotic-, intestinal or kidney arteries. The costs for up to nine months treatment in combination with ASA after PCI for acute myocardial infarction, are covered by Rikstrygdeverket/ NAV trygd.

The ADP- pathway of platelet aggregation
Basic intracellular signalling pathways:
Circulating platelets are activated when being exposed to collagen and/ or von Willebrand factor at the site of a plaque rupture or endothelial fissures.
Activation of platelets results in the release of two major soluble agonists, tromboxane A_2, which is produced from membrane phospholipids (arachidonic acid) by cyclo-oxygenase 1/ thromboxane syntethase enzymes, and ADP released from dense granules. There are two G-coupled protein receptors for the ADP- induced platelet activation: $P2Y_{12}$ and $P2Y_1$(1), respectively mediating adenylcyclase inhibition and mobilization of intracellular calcium stores (2). Activation of both receptors is needed for complete aggregation (1).

A description of the intricate intracellular pathways following ADP binding to the P2Y receptors is in short (Figure 1):
$P2Y_1$ is coupled to the Gq protein. The intracellular signalling pathways activate the phospholipase C, resulting in mobilization of intracellular calcium. This initiates a series of events resulting in platelet shape change and weak aggregation in response to ADP. This reseptor has a crucial role in the initiation of platelet activation induced by ADP or collagen (3).

The $P2Y_{12}$ receptor is responsible for the completion of the platelet aggregation in response to ADP. It plays a central role in amplification of the aggregation induced by all known platelet agonists, whatever their signalling pathway. And it sustains and stabilises a forming thrombus (3).

The $P2Y_{12}$ receptor is linked to the G_i protein. Normally, intact endothelium secretes prostacyclin that activates adenylcyclase and increases platelet cAMP levels. However, activation of the $P2Y_{12}$ receptor inhibits adenylcyclase activity and thereby decrease cAMP levels. A reduction in cAMP lower the activity of cAMP-dependant protein kinases which in turn, reduce the levels of phosphorylated vasodilator stimulated phoshoprotein (VASP), and reduces its protective effect on the activation of the GP IIb/IIIa receptor.
Flowcytometric analysis of VASP phosphorylation has been used as a test of $P2Y_{12}$ activity and clopidogrel resistance.
In summary: Activation of the $P2Y_{12}$ receptor results in a cascade of intracellular signalling events that take part in the final activation of the GP IIb/IIIa receptor, the amplification of platelet aggregation, the facilitation of granule release, and the stabilization of the platelet aggregate (2).

Clopidogrel metabolism:
Clopidogrel is a pro- drug being absorbed from the intestine and transported into the blood stream. In the liver the pro-drug is excessively converted by the hepatic cytochrom P450 isoenzymes (CYP3A4, CYP3A5 and 2C19). About 85% of the drug is hydrolyzed by esterases to an inactive carboxylic acid derivative. However a small fraction is converted into the intermediate metabolite 2-oxo-clopidogrel by cytochrome P450 monooxygenase. The instable 2-oxo-clopidogrel is non-enzymatically hydrolyzed to the active thiol metabolite that irreversibly binds to the cystein residues (cys17 and cys270) on the platelet $P2Y_{12}$ receptor resulting in a partial inhibition of the platelet aggregation, (1), (2), (4), (5), (6).

Mechanisms of clopidogrel:
The disulphide bridge between the thiol metabolite of clopidogrel and the cystein residues on the $P2Y_{12}$ receptor results in the effective blockade of ADP-induced platelet activation and aggregation. Through the ADP-blockade, clopidogrel also inhibits the expression of P-selectin and CD40 ligand, preventing the formation of heterotypic aggregates and induction of

inflammation. It is important to bear in mind that clopidogrel only partly inhibit the ADP activating pathway.

Although a platelet inhibitory effect has been detected in healthy volunteers as early as two hours after a 75mg dose of clopidogrel, a steady-state of inhibition (50-60%) was reached only after repeated administration of a daily dose of 75mg for 5-7 days (1). A loading dose between 300 and 600mg clopidogrel will help achieve steady-state faster, and is often used in clinical practise. (1).

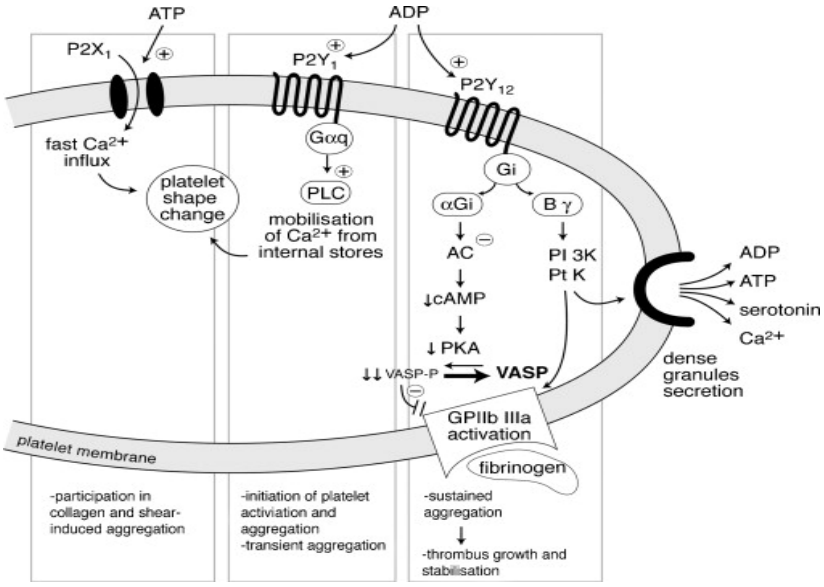

Figure 1. Adenosine diphosphate (ADP) and the $P2Y_{12}$ receptor. Activation of the $P2Y_{12}$ receptor by ADP liberates the G_i protein subunits αG_i and B_γ. αG_i inhibits adenylyl cyclase (AC) decreasing platelet cyclic adenosine monophosphate (cAMP) levels with the consequent reduction in protein kinase (PKA) phosphorylation of vasodilator-stimulated phosphoprotein (VASP-P). This results in a decrease in VASP-P inhibition of glycoprotein IIb/IIIa (GP IIb/IIIa), with the consequent GP IIb/IIIa activation and resultant platelet aggregation. Subunit B_γ activates phosphoinositol 3-kinase (PI 3K) and phosphotyrosine kinases (Pt K) linked to GP IIb/IIIa activation and platelet dense granule secretion. PLC: phospholipase C; ATP: adenosine triphosphate. **Oqueli et al. Clopidogrel resistance. Heart, Lung and Circulation 2007.**

Clopidogrel non- responsiveness

Currently there is no universally accepted definition of *clopidogrel non- responsiveness*, also called clopidogrel resistance, clopidogrel low responsiveness or hyporesponsiveness. Clopidogrel non- responsiveness will be the used term for the following.

In its broadest sense, non- responsiveness could refer to continued occurrence of ischemic events in spite of antiplatelet therapy with compliance. Gurbel et al define non-responsiveness as failure of the antiplatelet agent to inhibit the target of its action (6). Clopidogrel non- responsiveness might therefore be best demonstrated by evidence of residual

post- treatment $P2Y_{12}$ activity by measuring ADP- induced platelet aggregation before and after treatment, although ADP is not specific for $P2Y_{12}$- receptor activation.

The difference between pretreatment and posttreatment ADP- induced platelet aggregation has been used as an estimate of clopidogrel non- responsiveness (2), (7), (8), (9), (10). Clopidogrel non- responsiveness has in some studies (7), (8), (9) been defined as an absolute difference between baseline aggregation and posttreatment aggregation of 10% or less with 5 μmol/L ADP used as agonist, tested by turbidimetric platelet aggregometry. This is an in vitro evaluation of platelet aggregation, and the importance of this laboratory phenomenon on clinical endpoints is not yet established.
Current estimates on the frequency of clopidogrel non- responsiveness are between 5- 44% (6).

Platelet function testing
Current standard for platelet function testing is turbidimetric platelet aggregometry, despite of poor reproducibility, high sample volume, need of sample preparation, need of technicians, time, expense etc. New point- of- care tests for platelet function have been developed including VerifyNow (Accumetrics, San Diego, CA) and PFA-100 (Dade behring, Newark, DE) (10), (11).

Clopidogrel non- responsiveness: possible mechanisms
Non- compliance, and under- dosing or inappropriate dosing of clopidogrel are possible mechanisms of non- responsiveness. There are different opinions regarding the optimal loading and maintenance dose regimens. Several studies have shown that a 600 mg loading dose of clopidogrel gives a superior antiplatelet effect than a 300 mg loading dose for patients undergoing coronary stenting (8), (12). In the study of Gurbel et al, (8), the aim of study was to determine the effect of clopidogrel dosing on the incidence of non- responsiveness and high posttreatment platelet aggregation. They defined clopidogrel non- responsiveness as a < 10% change in aggregation (baseline aggregation minus posttreatment aggregation). In the 600 mg group, they had lower post treatment platelet aggregation, greater change in aggregation, and a lower percentage of non- responders compared to the 300mg group. They concluded that estimates of non- responsiveness are dose- dependent. In the ISAR- CHOICE study, (12), a loading dose of 900mg clopidogrel compared to a 600mg loading dose showed no further

increase in plasma concentrations of active metabolite, and no further suppression of ADP-induced platelet aggregation.

Failure of metabolization of clopidogrel into an active metabolite in the liver by CYP 3A4 could be the mechanism of non- responsiveness to the drug.
This can be caused by a variability in the phenotypic expression of hepatic CYP 3A4 (2),(13) and also by genetic polymorphisms of the CYP3A4 gene (14).
It can also be caused by drug- drug interactions. Many drugs are metabolized by CYP 3A4. Any drugs inhibiting or being a substrate of the CYP3A4 can potentially block the conversion of clopidogrel into its active metabolite (14). Atorvastatin is one of these drugs. Some studies have shown that atorvastatin do influence the level of clopidogrel induced platelet inhibition, other studies have concluded that this interaction is not clinically relevant (15). Schroeder et al sum up some of the studies on clopidogrel and HMG-CoA reductase inhibitors' interaction (16). An in vitro interaction between a CYP 3A4 substrate statin and clopidogrel has been shown, but the clinical relevance of this interaction has not been recognised. Studies point in this direction, but sample size makes it non- statistically significant. Their conclusion is that further prospective studies on the hard clinical events are needed (16).
Erythromycin is another drug that compete with clopidogrel for CYP3A4 metabolism.
Whether the drug rifampin and the herb St. John's wort enhance the inhibitory effect of clopidogrel by induction of the CYP 3A4 gene remains questionable (2), (6).
Impaired or variable gastrointestinal absorption of the prodrug is another possible mechanism of non- responsiveness.

Fontana et al argues that there is a large variability in the number of $P2Y_{12}$ receptors in individuals, and questions whether genetic variability of the $P2Y_{12}$ receptor could be a mechanism of non- responsiveness. Two haplotypes of the $P2Y_{12}$ receptor gene have been identified, the H1 and H2 haplotypes. The H2 haplotype is associated with maximal platelet aggregation in response to ADP, and therefore carriers with an increased number of the ADP $P2Y_{12}$- receptors associated with the H2 haplotype may have an increased risk of atherothrombosis and/ or a lesser clinical response to antiplatelet drugs (17).
Variability in the downstream intracellular signalling, or alternate pathways of platelet activation have also been suggested as mechanisms for non- responsiveness. For example by the increased extent of $P2Y_1$ receptor- dependent platelet aggregation or up- regulation of

other pathways of platelet activation (thrombin, thromboxane A2, collagen) (3), or yet another possibility: increased reactivity of resting platelets and high pretreatment platelet reactivity.

Present study

Aim of study

The aim of the present study was to define a cut off point for clopidogrel non- responsiveness in a study population of patients with stable coronary heart disease (CHD), and possibly give an estimation of the prevalence of non- responders in patients with stable CHD, evaluated by the recently developed method VerifyNow.

Methods

Study design

The patients were part of the Aspirin non- responsiveness and clopidogrel clinical endpoint trial (ASCET) which is a prospective randomized study primarily investigating aspirin non-responsiveness in relation to clinical events (18). Patients of both sexes with stable CHD verified with coronary angiography were before randomization given aspirin 160mg/d for at least one week (n= 227). They were then randomized to either continuing aspirin 160 mg daily or change to clopidogrel (Plavix®, Sanofi Winthrop Industries, Ambarese, France and Bristol-Myers Squibb, Paris, France) 75 mg daily. Both dosages are in accordance with current guidelines. Randomization was performed according to a computer- generated randomization list indicating the treatment allocation for each patient, developed by the Center for Clinical Research, Ullevål University Hospital, Oslo, Norway. Consecutively numbered sealed envelopes, containing treatment modality according to the randomization list, were used. Patients continued all other medication according to general guidelines.
The present investigation is based on a subpopulation of 227 consecutive enrolled patients of which 91 were randomized to clopidogrel.

Laboratory methods (VerifyNow)

At randomization standard baseline blood sampling was performed, at 8- 10 AM, in fasting condition without any medication by venipuncture (Vacutainer tubes containing 3,2% sodium citrate in dilution 1:10 (Gruner Bio- One GmbH, Kremsmünster, Austria)), and the samples were tested with VerifyNow $P2Y_{12}$ (n = 227). After 1 month patients randomized to clopidogrel treatment were retested with the VerifyNow $P2Y_{12}$ assay (n= 91).

The VerifyNow $P2Y_{12}$ Assay (Accumetrics, San Diego, CA) is designed to measure platelet $P2Y_{12}$ receptor blockade. It is developed to measure responsiveness to $P2Y_{12}$ receptor antagonists such as clopidogrel and prasugrel (19),(20). The VerifyNow system is a turbidimetric based optical detection system. It measures platelet- induced aggregation. The assay is based on the ability of activated platelets to bind fibrinogen. It contains microbeads coated with fibrinogen, aggregating in whole blood in proportion to platelet GPIIb/IIIa receptor expression, which increases when platelets are activated. The VerifyNow $P2Y_{12}$ assay uses ADP and PGE1 as activators of platelets in one assay channel (the ADP channel). In adding PGE1, the ADP- induced $P2Y_1$- mediated platelet activation is suppressed. Light transmittance increases when platelets bind and aggregate the fibrin- coated microparticles (the light transmittance is dependent on number of particles, not their size). The instrument measures the change in optical signal in PRU (Platelet Reaction Units). In addition to ADP/PGE1, a second activator (iso- TRAP or Thrombin Receptor Activation Peptide) and fibrinogen- coated microparticles are incorporated into a second channel of the assay device. The aggregation of platelets induced by iso- TRAP occur independent of the $P2Y_{12}$ receptors. The TRAP channel gives a measure of the baseline platelet function, BASE result. The BASE result is an estimate of the baseline platelet function. The results from both channels in the device are used to calculate percent inhibition in the sample. Percent inhibition is calculated as follows: % inhibition = ((BASE-PRU) x 100)/ BASE. The results given by the $P2Y_{12}$- assay can therefore be reported as both PRU- and % inhibition (19, 22).

Statistics

We performed individual statistics for the parameters PRU and % inhibition using the SPSS software, version 14.0. Only descriptive statistics were used.

Results

Patient characteristics at baseline

By a randomized selection of n≈ 200 from the ASCET study population, only few significant differences between the two groups were recorded at baseline, see table 1 (23). There were more frequent hypertension in the clopidogrel group (p= 0.04) and a greater number of PCI treated patients in the aspirin group (p=0.04). In accordance with current guidelines 98% were on statin therapy. About 20% were current smokers and 20% had known diabetes mellitus type II.

Table 1: Characteristics of a selection of the ASCET- study population at baseline. Proportion or median values (25, 75 percentiles) are given.

	Aspirin 160mg (n=105)	Clopidogrel 75mg (n=101)
Age, years	62 (56, 69)	61 (52, 79)
Sex, % female	22	19
Hypertension %[1]	48	60
Diabetes %	18	20
Smokers %	19	20
BMI kg/m^2	27.5 (24.8, 31.2)	27.5 (25.2, 29.3)
Previous AMI %	53	50
Previous PCI %[1]	46	33
SBP/DBP mmHg	140/85	140/85
Previous ACB %	25	16
Total cholesterol mM	4.6 (4.2, 5.3)	4.4 (4.0, 5.3)
LDL cholesterol mM	2.7 (2.2, 3.2)	2.5 (2.1, 3.0)
HDL cholesterol mM	1.2 (1.1, 1.5)	1.2 (1.1, 1,5)
Triglycerides mM	1.3 (0.9, 1.9)	1.4 (0.9, 1.9)
Medication		
Aspirin %	100	100
Clopidogrel %	0	0
ACE-I %	29	37
AII antagonist %	22	21
β-blocker %	82	77
Ca- blocker %	31	35
Statin %	97	98

BMI: body mass index, AMI: acute myocardial infarction, PCI: percutaneous coronary intervention, SBP: systolic blood pressure, DBP: diastolic blood pressure, ACB: aortocoronar bypass, ACE-I: angiotensin-converting enzyme inhibitor, AII antagonist: angiotensin II receptor antagonist, β- blocker: beta- adrenergic receptor blocker, Ca- blocker: calsium- channel blocker. [1]p=0.04 for difference between groups.

Baseline results

227 patients were tested with the VerifyNow $P2Y_{12}$ assay at baseline, for 5 of the patients results are missing due to failure of analysis (error).

% inhibition results

Table2: distribution of results

N	Valid	222
	Missing	5
Mean		7,97
Median		6,00
Std. Deviation		7,949
Percentiles	25	,00
	50	6,00
	75	13,00
	95	23,70

Expected values of inhibition of the $P2Y_{12}$-reseptor at baseline were low, close to zero as all patients were on aspirin and not on treatment with clopidogrel at this point.
Median value was 6, which indicated that the majority of the patients had no inhibition, as anticipated. 95% of the patients had 24% inhibition or less of the $P2Y_{12}$- receptors as shown in the 95 percentile. We chose this to be our cut-off value. The distribution is also shown in fig 2, the cut off level indicated with an arrow.

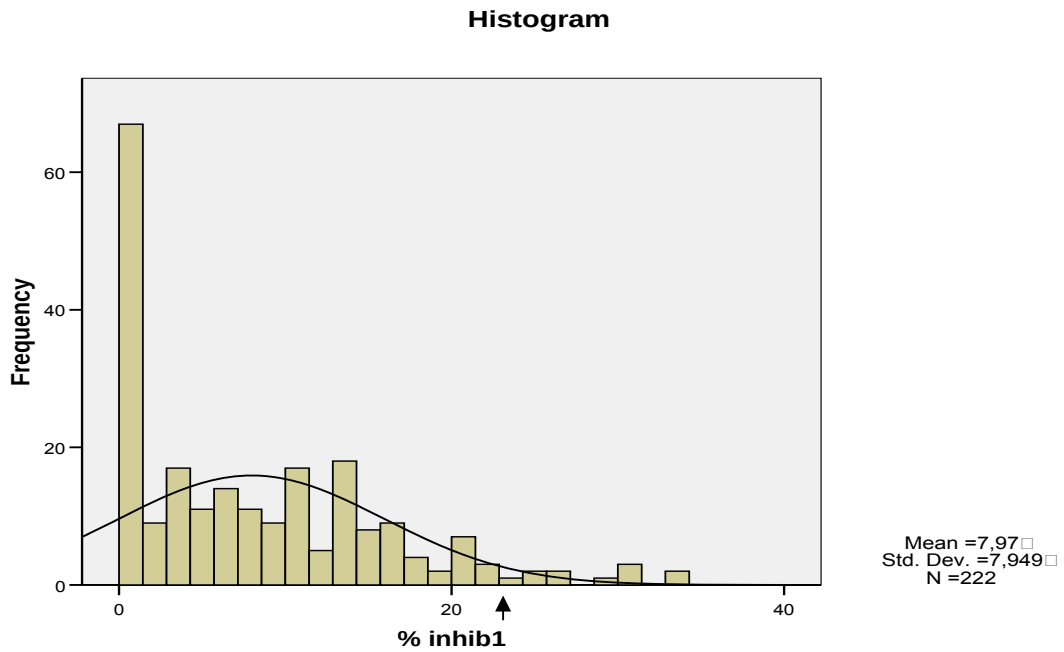

Fig 2

PRU- results

Table 3a: Distribution of results

N	Valid	222
	Missing	5
Mean		243,69
Median		240,00
Std. Deviation		45,211
Percentiles	25	212,00
	50	240,00
	75	276,50
	95	316,85

Table 3b

		Percentiles						
		5	10	25	50	75	90	95
Weighted Average(Definition 1)	PRU 1	168,75	184,00	212,00	240,00	276,50	299,70	316,85
Tukey's Hinges	PRU 1			212,00	240,00	276,00		

Expected values of platelet reactivity (measured in units) in persons non-inhibited (not on clopidogrel treatment) are high.

The median value was 240. The 5% percentile of PRU was approximately 170, see table 3b. We defined a PRU- value of less than 170 as "good inhibition" or responsiveness. The results are also shown in fig 3, where a cut-off point of 170 (corresponding to a 5% percentile of PRU) is shown by arrow.

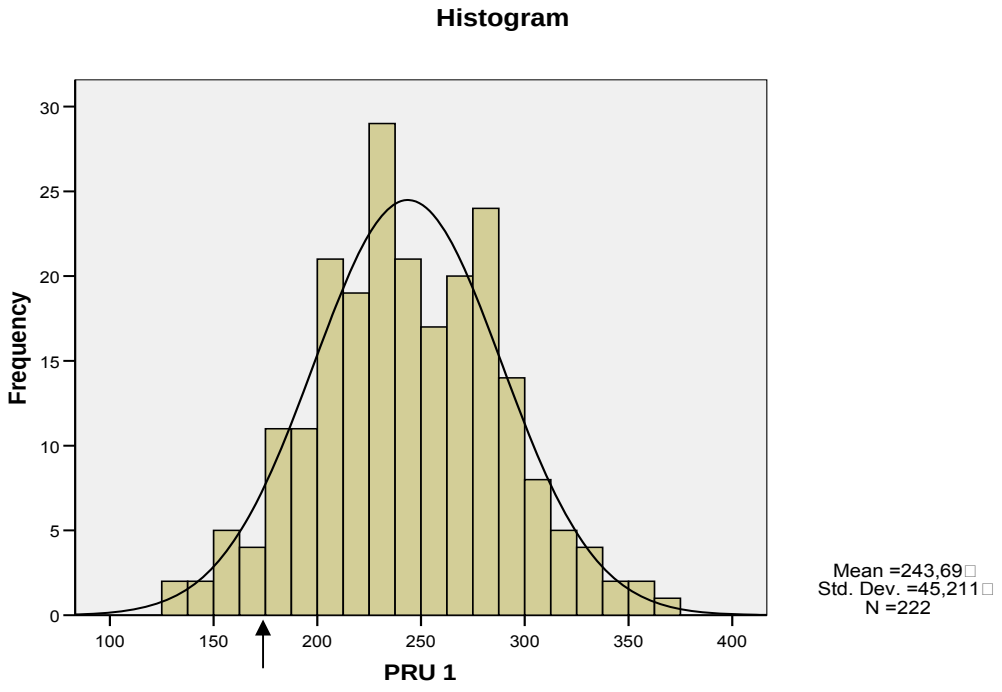

Fig3

After one month on Clopidogrel treatment

% inhibition results

Table 4: Distribution of results

N	Valid	88
	Missing	2
Mean		41,78
Median		43,00
Std. Deviation		24,661
Percentiles	25	19,00
	50	43,00
	75	56,75
	95	89,20

91 patients were randomized to clopidogrel treatment. Due to one failure of recording of % inhibition, statistics were performed on n = 90. For 2 patients results were missing due to failure of analysis.

Median % inhibition was 43. Given our cut-off value of 24% defined at baseline, we considered all patients with a treatment response with value 24% and less to be non-responders to clopidogrel. The cumulative percent of non- responders was 28%. The results are also shown in fig 4, arrow indicating the cut- off value.

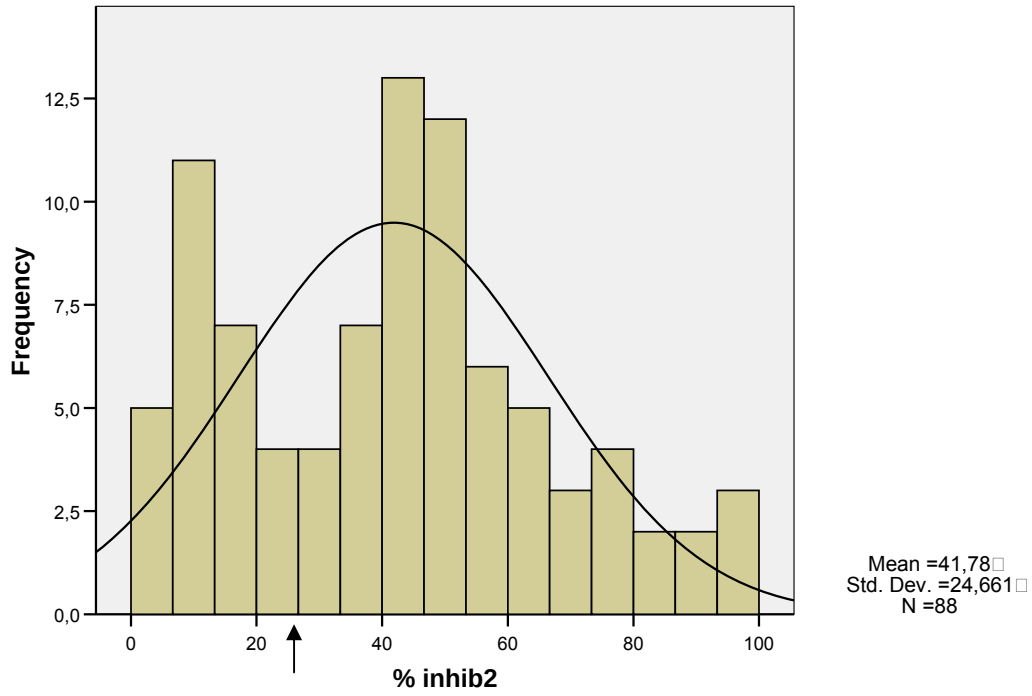

Fig 4

PRU- results

Table 5: Distribution of results

N	Valid	89
	Missing	2
Mean		139,60
Median		140,00
Std. Deviation		67,132
Percentiles	25	86,00
	50	140,00
	75	195,50
	95	243,50

Median value for PRU after one month was 140. From the statistics performed at baseline the cut off value of 170 was defined for non- responsiveness. When we looked at the cumulative percent, there were approximately 35% non- responders (having a PRU- value of 170 or more).

Results are also shown in fig 5, arrow indicating the cut- off level. The histogram for PRU shows an approximately normal distribution.

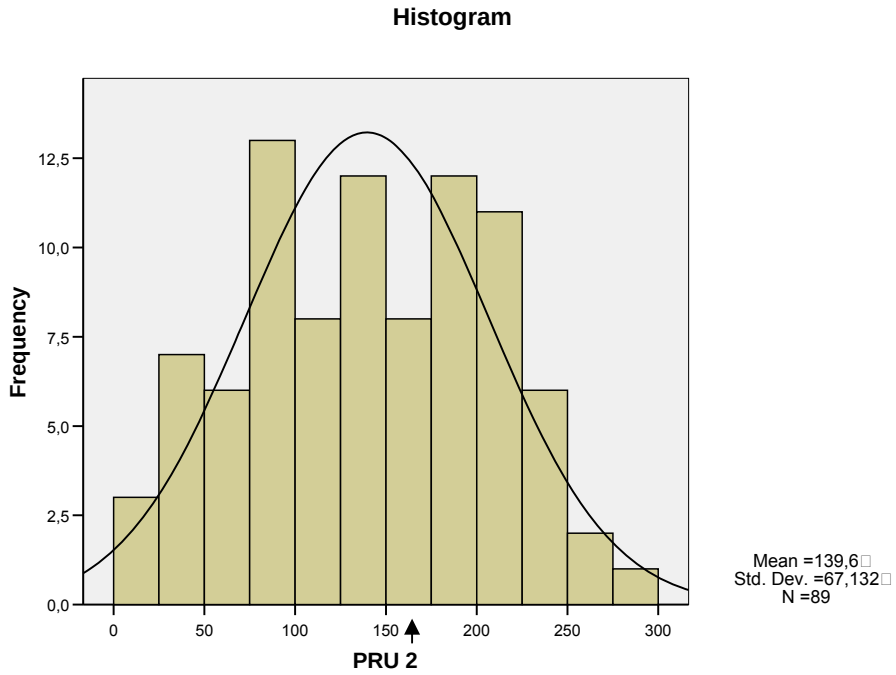

Fig 5

Changes from baseline to one month on treatment

The changes in % inhibition are shown in fig. 6, a comparison of the same individuals at baseline and after one month on clopidogrel treatment. Median % inhibition changed from 6% to 43%.

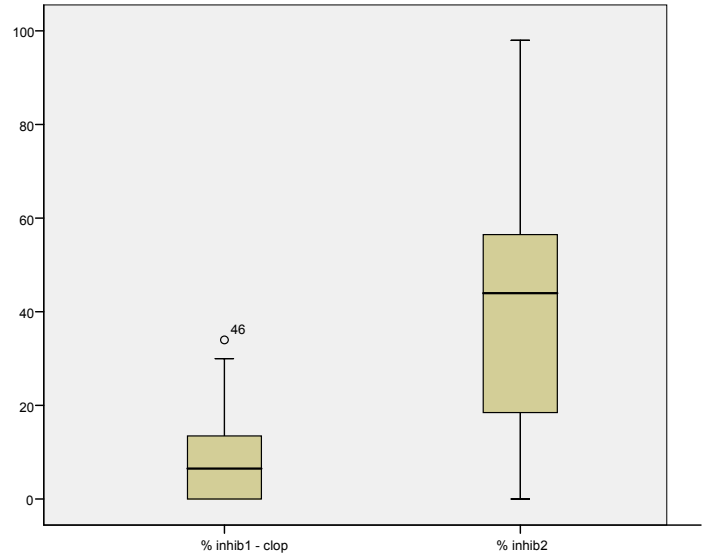

Fig 6

Changes in PRU from baseline to one month on clopidogrel treatment are shown in fig. 7. Decrease in median PRU from 240 to 140.

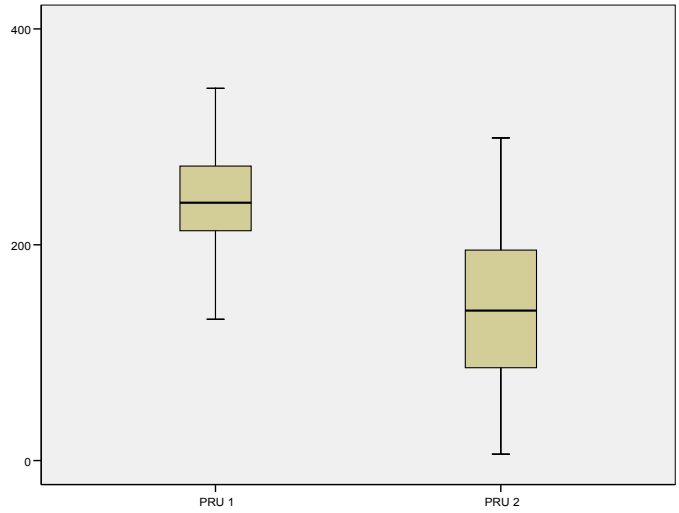

Fig 7

Comparison of the different ways to express the results (PRU and % inhibition)

The 35% (PRU) - and the 28% (% inhibition) non- responders does not represent a great difference. We have checked if the 28% non responders defined by % inhibition, were included in the 35% defined by measuring PRU.

There were 2 individuals who were non- responders with the % inhibition and not with the PRU. The other non- responders with % inhibition were all included amongst the 35% non responders with PRU.

Discussion

The aim of our study was to estimate the prevalence of clopidogrel non- responders in a given population of patients with CHD. We investigated two variables, PRU and % inhibition, given from the VerifyNow screening method.

PRU and % inhibition display close to opposite results: A high PRU value indicates high platelet reactivity. This is confirmed by a low % inhibition value.

At baseline that was exactly what we found. A histogram of PRU showed normal distribution of the material.

This result is reflected in % inhibition, with median value of 6. A low value of % inhibition was expected at this point, where no clopidogrel treatment was initiated.

The 5% percentile of PRU at baseline (PRU1) was close to 170, and we used this as our "cut-off- point" corresponding to a commonly used limit with 5% of a reference population being regarded as "irregular". After one month on treatment a PRU value less than 170 were regarded as responsiveness to clopidogrel. The equivalent value in % inhibition is the 95% percentile which was 24%. Our "reference population" was CHD patients on ASA, thought to be relevant.

After one month on clopidogrel treatment new analyses were performed. When applying the cut off value on these results, we calculated an estimation of prevalence of non responsiveness to clopidogrel for PRU and % inhibition of 35% and 28%, respectively. Comparing the individuals in each group we found 2 individuals who were non- responders with the % inhibition and not with the PRU. The other non- responders with % inhibition were all included amongst the 35% non- responders with PRU. This gives support to the validation of the VerifyNow.

The VerifyNow $P2Y_{12}$ assay is a relatively newly developed method for measuring platelet reactivity. A few studies have been performed using this assay, for example the VERITAS-study that indicates that the assay is a sensitive device for measuring platelet inhibition with clopidogrel (22). Van Werkum et al have compared the VerifyNow Assay to light transmittance aggregometry for assessing the inhibitory effects of clopidogrel on platelet function. They found a strong correlation between the two methods of measurement looking at late aggregation and PRU (20).
The assay automatically displays the results in PRU, and to retrieve the % inhibition result a manual operation has to be performed. There is a possibility of miscalculation of % inhibition in the assay. % inhibition is a calculated result, based on analysis in both assay- channels. An error in either assay- channel will give a false result.
Ex vivo platelet function tests do not necessarily give an accurate measurement of in vivo platelet function. Other factors (not measured) in vivo may influence platelet reactivity, and thrombus formation. Laboratory non- responsiveness may not be the same as clinical non-responsiveness and vice versa.

Snoep et al has given a systematic review and performed a meta- analysis on clopidogrel non-responsiveness in patients undergoing PCI with stenting (24). They found the mean prevalence of laboratory clopidogrel non-responsiveness to be 21%. They also found that laboratory non- responsiveness to clopidogrel was associated with *"clinical non-responsiveness"* (increased risk of stent thrombosis and other cardiovascular outcomes). The time of blood sampling after loading of clopidogrel could be a reason for differences in prevalence of non- responsiveness during clopidogrel treatment. Reported prevalences decreased with increasing time to blood sampling after loading. In one study of clopidogrel non- responsiveness in patients undergoing elective stenting who received a 300 mg loading dose at the time of stenting, 63% of patients were non- responders at 2 hours, approximately 30% at days 1 and 5 post stenting, and 15% by day 30 on continuous maintenance dosing (7). According to Snoep et al the same effect of time on prevalence of non- responders has not been shown when a 600mg loading dose has been used, given identical maintenance dosing (24).

A 600 mg loading dose of clopidogrel has proved superior to a 300mg loading dose in acute disease states. It gives a lower prevalence of non- responders and studies have indicated a

lower incidence of cardiovascular events. This needs verifying in prospective studies (6), (24). The factor of loading dose does not apply to our study, due to already reached steady- state of inhibition after one month on clopidogrel treatment.

There are limited data available to link clopidogrel non- responsiveness to the occurrence of thrombotic events (6). The review article of Siller- Matula et al comments the discrepancy between the high rate of clopidogrel non- responsiveness (up to 40%), and the corresponding quite low overall incidence of acute and subacute stent thrombosis (0,6- 2%) (25). In this article they also comment on late stent thrombosis, occurring months after stent implantation. Combined antiplatelet therapy is often reduced 6- 12 months after initiation. This has been suggested as reason for late thrombosis. The authors suggest pathological mechanisms and failure of complete reendothelialization in the vessel walls as main reasons for this phenomenon. This hypothesis is strengthened by the observation of thrombosis not necessarily occurring immediately after stopping clopidogrel treatment, but up to months later (25).

Increased loading dose (600mg) of clopidogrel could reduce the prevalence of non-responders to the drug, but not eliminate the problem. Research is ongoing on new thienopyridines, not currently on the market. Prasugrel is one of the most promising, and phase two studies on healthy volunteers have already been performed with optimistic results. Phase one trials on patients with coronary heart disease give similar results. Prasugrel is an orally active drug, it is completely converted into its active metabolite, meaning no metabolic waste. Prasugrel is up to ten times more potent than clopidogrel. Siller- Matula et al describes early data from the phase three study TRITON TIMI 38, which compare clopidogrel with prasugrel in STEMI or NSTEMI patients. The results indicate that prasugrel produces a more consistent platelet inhibition compared with clopidogrel. In the group treated with clopidogrel there was a percentage of non- responders of 22- 43%, whereas in the prasugrel- group there were none (3), (25). Recently, the final clinical report from this study indicate improved outcome in cardiovascular end- points on the cost of significantly more bleeding episodes (26).

Finally, the ASCET study (18) will hopefully give additional knowledge about the relation between clopidogrel non- responsiveness in the laboratory and relevant clinical end- points.

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