Platelet reactivity and cardiovascular events

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CLOPIDOGREL NON-RESPONSIVENESS IN PATIENTS UNDERGOING PERCUTANEOUS CORONARY INTERVENTION WITH STENTING: A SYSTEMATIC REVIEW AND META-ANALYSIS

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American Heart Journal 2007;154:221-231

ABSTRACT

Background

Despite clopidogrel therapy, patients undergoing percutaneous coronary intervention (PCI) with stenting are at risk of recurrent coronary events. This could be partly explained by a reduced efficacy of clopidogrel to inhibit platelet aggregation, an ex vivo defined phenomenon called clopidogrel non-responsiveness or resistance. However, both prevalence and associated cardiovascular risks remain unclear. We systematically reviewed evidence on prevalence and clinical consequences of laboratory clopidogrel non-responsiveness in patients undergoing PCI.

Methods

Using predefined strategies, we searched electronic databases. To be included, articles should report on PCI patients treated with clopidogrel, contain a clear description of the method used to establish the effects of clopidogrel, and report the prevalence of clopidogrel non-responsiveness or incidence of cardiovascular events. We analyzed prevalences with a linear mixed model that accounts for study covariates and we pooled odds ratios of clinical consequences with a random-effects model.

Results

We identified 25 eligible studies that included a total of 3688 patients. Mean prevalence of clopidogrel non-responsiveness was 21% (95%CI, 17% to 25%) and was inversely correlated with time between clopidogrel loading and determination of non-responsiveness and used loading dose. The pooled odds ratio of cardiovascular outcome was 8.0 (95%CI, 3.4 to 19.0).

Conclusions

Laboratory clopidogrel non-responsiveness can be found in approximately 1 in 5 patients undergoing PCI. Patients ex vivo labeled non-responsive are likely to be also "clinically non-responsive," as they exhibit increased risks of worsened cardiovascular outcomes. Our results indicate that use of a 600-mg clopidogrel loading dose will reduce these risks, which needs to be confirmed in large prospective studies.

INTRODUCTION

Cardiovascular diseases are the most common cause of mortality and morbidity in Western countries in the twenty-first century. In patients with symptomatic coronary artery disease, percutaneous coronary intervention (PCI) with stenting is effective to prevent further ischemic events.[1,2] However, recurrent coronary events, including stent thrombosis, remain a serious complication of this procedure and are associated with increased morbidity and mortality.[3-5] To prevent recurrent events, clopidogrel is currently routinely added to aspirin in treatment of patients undergoing coronary stenting.[6] This thienopyridine is oxidized by the hepatic cytochrome P450 to an active metabolite and can irreversibly block the adenosine diphosphate (ADP) receptor P_2Y_{12},[7] which plays an important role in platelet activation.[8]

The clinical effectiveness of addition of clopidogrel to aspirin therapy to prevent cardiovascular events after PCI has been shown repeatedly.[9-11] However, not all patients profit to the same extent, which may be partly explained by a reduced efficacy of clopidogrel to inhibit ADP-induced platelet activation through blockade of the P_2Y_{12} receptor.[12] When addressed biochemically as failure to inhibit platelet function *ex vivo*, this phenomenon is called clopidogrel non-responsiveness, low responsiveness or resistance.[13-15]

Prevalences of laboratory-defined clopidogrel non-responsiveness vary widely in literature.[13,16] Furthermore, the main question as to whether patients that are biochemically labeled clopidogrel non-responsive also exhibit "clinical non-responsiveness" to clopidogrel, i.e., a higher risk of stent thrombosis and other recurrent ischemic events, remains largely unanswered hitherto. To try to quantify evidence addressing these topics, we conducted a systematic review and meta-analysis of all reports on both prevalence and clinical consequences of laboratory clopidogrel non-responsiveness among patients undergoing PCI with stenting.

METHODS

We searched MEDLINE (from January 1966 until October 2006), EMBASE (from January 1974 until October 2006), the Cochrane Central Register of Controlled trials (CENTRAL) (from 1800 until October 2006), and Web of Science (from 1945 until October 2006), using predefined search terms (available from the authors). We used no language restrictions. Furthermore, we searched reference lists of relevant studies and reading reviews, editorials, and letters on this topic. Authors of identified appropriate studies were contacted to obtain additional data not reported in the original report. Both full-text articles and meeting abstracts were included.

To be included, selected studies had to meet the following inclusion criteria: (1) involved patients should use clopidogrel to prevent coronary events after PCI with stenting; (2) the study should contain a clear description of the laboratory method used to establish the effects of clopidogrel on platelet reactivity; and (3) the report should supply data either on prevalence of laboratory clopidogrel non-responsiveness

or on occurrence of stent thrombosis (subacute) or other ischemic events as predefined by investigators, or both.

The quality of the identified studies was assessed based largely on quality criteria concerning minimization of bias. In detail, we evaluated information regarding control for confounders, measurement of exposure, completeness of follow-up, and blinding. For case-control studies, we also assessed matching and case definition. No formal scoring system was used. Reviewers were not blinded to journal, author, or institution of publication.

We used a prespecified data collection form to extract information for each report regarding year of publication, study duration, design, sample size, and population (baseline characteristics). Concerning our research questions, we collected the following variables: clopidogrel loading and maintaining dose, concomitant aspirin dose, definition of non-responsiveness, time between clopidogrel loading and determination of non-responsiveness, prevalence of non-responsiveness, definition and incidence of clinical outcomes.

Selection, quality assessment, and data extraction of studies to be included in this review were all independently done by 2 reviewers (M.M.C.H. and J.D.S.). Disagreements were resolved by consensus and discussion with a third party (M.V.H.). For agreement between reviewers, κ statistics were calculated manually for each process in study selection. The overall κ was calculated as a weighted mean of those values.

To estimate the pooled prevalence of clopidogrel non-responsiveness, we stratified studies based on method used to assess non-responsiveness, time between clopidogrel loading and determination, clopidogrel loading dose, and concomitant dose of aspirin. We defined 3 groups of studies based on method used: (1) light transmittance aggregometry (LTA) using 5 μmol/L ADP as agonist, (2) aggregometry with 20 μmol/L ADP, and (3) other methods. In our third group, we categorized few studies using other techniques (flow cytometry of platelet-bound fibrinogen (after ADP stimulation) or vasodilator-stimulated phosphoprotein phosphorylation, whole-blood impedance aggregometry and LTA with other ADP concentrations). Time between clopidogrel loading and determination of non-responsiveness was determined in 4 groups: (1) <24 hours, (2) 24 to 48 hours, (3) 2 to 7 days, and (4) >7 days. We classified 2 different clopidogrel loading doses (300 and 600 mg) and we assessed the potential influence of concomitant use of aspirin on clopidogrel non-responsiveness in 4 groups: (1) ≤100 mg, (2) 101 to 300 mg, (3) ≥300 mg and (4) no specified dose of aspirin.

To relate laboratory clopidogrel non-responsiveness to clinical outcomes, we calculated crude odds ratios (ORs) with 95% CIs for each study that reported proportions of responsive and non-responsive patients with cardiovascular events. We pooled ORs for non-responsiveness both from all eligible studies reporting cardiovascular outcomes and from several subgroups of studies. These subgroups included studies reporting on occurrence of (1) stent thrombosis; (2) a composite end point of clinical ischemic events, including cardiovascular death, myocardial infarction, stroke, acute coronary syndrome, revascularization, and stent thrombosis; and (3) myonecrosis after PCI represented by creatine kinase (CK)-MB elevation. We also calculated the positive

predictive value (PPV, chance of events in case of laboratory non-responsiveness) and negative predictive value (NPV, chance of remaining event-free in case of laboratory responsiveness) of non-responsiveness.

Statistical analysis regarding prevalence of non-responsiveness was based on a general linear nonparametric mixed model, which is a meta-analytical approach to explain heterogeneity among studies by modeling for available study covariates.[17,18] We performed this mixed model analysis for prevalence of clopidogrel non-responsiveness both with and without fixed effects for laboratory method used to determine non-responsiveness, time between clopidogrel loading and determination, and loading dose of clopidogrel and concomitant dose of aspirin, and with an identification number for each study as random effect. We also performed multivariate linear regression analyses to examine which factors influenced prevalence. Odds ratios of cardiovascular outcome were pooled using a random-effects model.[19,20] This rather conservative method for meta-analysis partly accounts for the possibility of statistical inter-study heterogeneity.

Both for studies on prevalence and on clinical consequences of non-responsiveness, we tested for statistical inter-study heterogeneity using random-effects models. For prevalence data we used generic inverse variance data entry. The χ^2-value was calculated for the hypothesis of homogeneity. Heterogeneity was quantified by means of I^2, which demonstrates the percentage of total variation across studies due to heterogeneity.[21]

Analyses were performed with SPSS 14.0.0 (SPSS Inc., Chicago, IL, USA) and Review Manager 4.2.9 (Cochrane Library Software, Oxford, UK).

RESULTS

By subsequent screening and assessment of titles, abstracts, and full-text articles, we included twenty-five studies that incorporated a total of 3688 patients (Figure 1). Nineteen full-text articles[13-16,22-36] and three meeting abstracts[37-39] (2574 patients) addressed the prevalence of laboratory clopidogrel non-responsiveness in patients undergoing PCI, whereas clinical consequences were studied in ten full-text articles[14,25,31-34,36,40-42] and one meeting abstract[38] (2319 patients). Details of included studies are summarized in Tables 1 and 2. Overall, κ statistics for agreement between reviewers were 0.91.

Prevalence

Among patients undergoing PCI with stenting, the mean unadjusted prevalence of clopidogrel non-responsiveness, weighted for study size, was 21% (95%CI 17 to 25%). Table 3 presents pooled prevalences, both unadjusted and adjusted for time between clopidogrel loading and determination of non-responsiveness, laboratory method used, loading dose of clopidogrel, and dose of aspirin. There was significant heterogeneity among studies (I^2=90.5, χ^2=472.66, P<0.0001).

Table 1 – Details of included studies on prevalence of laboratory clopidogrel non-responsiveness

Investigators	Design	n	Clopidogrel dose (mg)	Aspirin dose (mg)	Definition of non-responsiveness
Jaremo *et al.*, 2002[22]	Prospective cohort	18	LD 300 MD 75	unknown	Flow cytometry of platelet bound fibrinogen (1.7 μM ADP induced) PR>60% of baseline
Mueller, *et al.*, 2002[37]	Prospective cohort	300	LD 600 MD 75	100	LTA (5 or 20 μM ADP): <20% reduction compared with baseline
Gurbel *et al.*, 2003[13]	Prospective cohort	92	LD 300 MD 75	81-325	LTA (5 μM ADP): <10% reduction compared with baseline
Gurbel *et al.*, 2003[23]	Prospective cohort	67	LD 300 MD 75	At least 81	LTA (5 μM ADP): <10% reduction compared with baseline
Gurbel *et al.*, 2003[24]	Prospective cohort	68	LD 300 MD 75	325	LTA (5 μM ADP): <10% reduction compared with baseline
Matetzky *et al.*, 2003[25]	Prospective cohort	60	LD 300 MD 75	200	LTA (5μM ADP): first quartile of reductions compared with baseline (least reduced)
Müller *et al.*, 2003[14]	Prospective cohort	105	LD 600 MD 75	100	LTA (5 or 20 μM ADP): <10% reduction compared with baseline
Angiolillo *et al.*, 2004[16]	Prospective cohort	50	LD 300 (n=27), 600 (n=23), MD 75	250	LTA (6 μM ADP): <10% reduction compared with baseline
Grossmann *et al.*, 2004[26]	Prospective cohort	57	LD 300 MD 75	100	Flow cytometry of VASP phosphorylation: PR>50%
Gurbel *et al.*, 2004[27]	Prospective cohort	94	LD 300 MD 75	325	LTA (20 μM ADP): higher reactivity compared with baseline
Hochholzer *et al.*, 2004[39]	Prospective cohort	78	LD 600 MD 75	At least 100	LTA (5 μM ADP): <10% reduction compared with baseline
Klamroth *et al.*, 2004[38]	Case-control	40	LD 300 MD 75	100	LTA (20 μM ADP): aggregation >30%
Lau *et al.*, 2004[28]	Prospective cohort	32	LD 300 MD 75	At least 81	LTA (5 μM ADP): <10% reduction compared with baseline
Angiolillo *et al.*, 2005[15]	Prospective cohort	48	LD 300 MD 75	250	LTA (6 μM ADP): <40% reduction compared with baseline
Dziewierz *et al.*, 2005[29]	Prospective cohort	31	LD 300 MD 75	75-100	LTA (20 μM ADP): <10% reduction compared with baseline
Gurbel *et al.*, 2005[30]	Randomized controlled trial	190	LD 300 (n=138), 600 (n=52), MD 75	325	LTA (5 and 20 μM ADP): <10% reduction compared with baseline
Wenaweser *et al.*, 2005[31]	Case control	73	LD 300 MD 75	100	LTA (5 μM ADP): <10% reduction compared with baseline
Cuisset *et al.*, 2006[32]	Prospective cohort	106	LD 300 MD 75	160	LTA (10 μM ADP): fourth quartile of aggregation
Cuisset *et al.*, 2006[33]	Randomized controlled trial	292	LD 300 n=146), 600 (n=146), MD 75	160	LTA (10 μM ADP): aggregation >70%
Geisler *et al.*, 2006[34]	Prospective cohort	379	LD 600 MD 75	100	LTA (20 μM ADP): aggregation >70%
Ivandic *et al.*, 2006[35]	Prospective cohort	244	LD 75-600 MD 75	unknown	Electrical aggregometry (5 μM ADP): 6-min impedance > 5Ω
Lev *et al.*, 2006[36]	Prospective cohort	150	LD 300 MD 75	81-325	LTA (5 and 20 μM ADP): <10% reduction compared with baseline with both agonists

ADP: adenosine diphosphate; LD: loading dose; LTA: light transmittance aggregometry; MD: maintenance dose; PR: platelet reactivity; VASP: vasodilator-stimulated phosphoprotein.

Moment of determination	Non-responsiveness, n (%)	Comments
24 h after LD	5 (28)	Small sample size Aspirin dose unknown
4 h after LD	48 (16) - 72 (24)	No information on patients (meeting abstract) Exclusion criteria not reported
2h, 24h, 5 d and 24 d after LD	58 (63), 28 (30), 28 (30), 14 (15)	
5 d after LD	16 (24)	No information on patients (short communication)
5 d after LD	16 (24)	No information on patients (letter) Exclusion criteria not reported
6 d after LD	15 (25)	Exclusion criteria not reported First quartile resistant: per definition 25% resistant
4 h after LD	5 (5) - 12 (11)	Exclusion criteria not reported
4, 24, 48 h after LD	300mg: 7 (26), 3 (11), 2 (7) 600 mg: 4 (17), 1 (4), 0 (0)	Small sample size Allocation to 300 and 600 mg LD not randomized
2-53 d after LD (median 5 d)	10 (18)	Exclusion criteria not reported. Platelet reactivity determined once and not at the same moment
2h, 24h, 5 d and 24 d after LD	52 (55), 24 (26), 20 (21), 14 (15)	
24 h after LD	33 (43), 17 (22)	No information on patients (meeting abstract) Exclusion criteria not reported
4 wk after LD	10 (25)	Aggregation determined once, Exclusion criteria not reported. No information on patients (meeting abstract)
5 d after LD	15 (30)	No information on patients Small sample size
24 h after LD	21 (44)	Small sample size
24 h after LD	7 (23)	Small sample size
24 h after LD	300 mg: 39-44 (28-32); 600 mg: 4 (8)	Significantly more patients with 600 mg LD were treated with glycoprotein IIb/IIIa inhibitors
31±4d after LD	4 (5)	Platelet aggregation not determined at the same time for each patient
12 h after LD	23 (22)	Not all patients received a loading dose Aggregation determined once
12 h after LD	300 mg: 36 (25), 600 mg: 22 (15)	Aggregation determined once
34.8±25.9 h after LD	22 (6)	Aggregation determined once
12-24 h after LD	40 (16)	No standardized LD. Responders median LD 450 mg vs. 300 mg non-responders
20-24 h after LD	36 (24)	

Table 2 – Details of included studies on clinical consequences of laboratory clopidogrel non-responsiveness

Investigators	Design	n	Clopidogrel dose (mg)	Aspirin dose (mg)	Definition of non-responsiveness	Moment of determination
Müller *et al.*, 2003[14]	Prospective cohort	105	LD 600 MD 75	100	LTA (5 or 20 μM ADP): <10% reduction compared with baseline	4 hours after LD
Matetzky *et al.*, 2003[25]	Prospective cohort	60	LD 300 MD 75	200	LTA (5μM ADP): first quartile of reductions compared with baseline (least reduced)	6 days after LD
Klamroth *et al.*, 2004[38]	Case-control	20 cases 20 controls	LD 300 MD 75	100	LTA (20 μM ADP): aggregation >30%	4 weeks after LD
Gurbel *et al.*, 2005[40]	Case-control	20 cases 100 controls	LD 300 MD 75	81-325	LTA (5 or 20 μM ADP): post treatment PR>75th percentile in controls	Cases 218±204 days after LD; Controls: 5-14 days after LD
Gurbel *et al.*, 2005[41]	Prospective cohort	192	LD 300 (n=75), 600 (n=60) MD 75	81-325	LTA (20 μM ADP): fourth quartile of aggregation	24 hours after LD
Wenaweser *et al.*, 2005[31]	Case-control	23 cases 50 controls	LD 300 MD 75	100	LTA (5 μM ADP): <10% reduction compared with baseline	31±4days after LD
Cuisset *et al.*, 2006[32]	Prospective cohort	106	LD 300 MD 75	160	LTA (10 μM ADP): fourth quartile of aggregation	12 hours after LD
Cuisset *et al.*, 2006[33]	Randomized controlled trail	292	LD 300 (n=146), 600 (n=146) MD 75	160	LTA (10 μM ADP): aggregation >70%	12 hours after LD
Geisler *et al.*, 2006[34]	Prospective cohort	379	LD 600 MD 75	100	LTA (20 μM ADP): aggregation >70%	34.8±25.9 hours after LD
Hochholzer, *et al.*, 2006[42]	Prospective cohort	802	LD 600 MD 75	≥100	LTA (20 μM ADP). No definition of non-responsiveness	At least 2 hours after LD
Lev *et al.*, 2006[36]	Prospective cohort	150	LD 300 MD 75	81-325	LTA (5 and 20 μM ADP): < 10% reduction compared with baseline with both agonists	20-24 hours after LD

ACS: acute coronary syndrome; ADP: adenosine diphosphate; CI: confidence interval; CK-MB: creatine kinase-myocardial band, CV: cardiovascular; LD: loading dose; LTA: light transmittance aggregometry; MD: maintenance dose; PAD: peripheral artery disease; PR: platelet reactivity; SAT: subacute stent thrombosis; ST: stent thrombosis; STEMI: ST-segment elevated myocardial infarction.

Non-responsiveness, n (%)	Endpoint	Follow-up	Clinical consequences, resistant vs. non-resistant patients	Comments
5 (5) -12 (11)	SAT	14 d	2 (16%) vs. 0 (0%) OR 44.5, 95%CI 2.0-991.0	Not clear whether SATs occurred in patients resistant with 5 or 20 μM ADP Adjudication (few) endpoints not blinded
15 (25)	STEMI, ACS, PAD ischemic stroke	6 m	7 (47%) vs. 1 (2%) OR 38.5, 95%CI 4.2-356.8)	Relatively small sample size Endpoints were not predefined
10 (25)	SAT	4 w	Cases vs. controls: 9 (45%) vs. 1 (5%) resistant OR 15.6, 95%CI 1.7-139.7	Aggregation determined once No information on patients (meeting abstract) Adjudication endpoints unblinded
Not reported	SAT	Cases: 218±204 d; Controls: 5-14d	Mean PR in cases was 49-65% vs. 33-51% in controls, p<0.001 for both 5 and 20 μM ADP	PR determined once PR measured at different times in cases and controls (later in cases). Significantly more drug-eluting vs. bare-metal stents in control group vs. cases
Not reported	CV death, MI, ACS, stroke	6 m	Mean post treatment PR 63±12 in patients with events (38, 20%) vs. 56±16 in patients without events (p=0.02).	57 were already on clopidogrel and did not receive a loading dose Allocation to 300 or 600 mg LD not randomized
4 (5)	SAT	>1 m	Cases vs. controls: 1 (4%) vs. 3 (6%) resistant OR 0.7, 95%CI 0.1-7.2	Small sample size Exact duration of follow-up not reported
23 (22)	CV death, ST, SAT, ischemic stroke, ACS	1 m	9 (39%) vs. 3 (4%) OR 17.1, 95%CI 4.1-71.3	Not all patients received a loading dose
58 (20) 15% LD 600 mg) vs. 25% LD 300 mg	CV death, ST, SAT, ischemic stroke, ACS	1 m	18 (31%) vs. 7 (3%) OR 14.6, 95%CI 5.7-37.2) 7 (5%) in LD 600 mg vs. 18 (12%) in LD 300 mg (p=0.02)	Aggregation determined once
22 (6)	CV death, MI, stroke	3 m	5 (23%) vs. 19 (6%) OR 5.0, 95%CI 1.7-15.0)	Aggregation determined once
Not reported	Death, MI, revascula-rization	1 m	1 (0.5%), 1 (0.5%), 6 (3.1%) and 7 (3.5%) events in 4 quartiles. 4th quartile highest aggregation	Aggregation determined once Relatively few events
36 (24)	CK-MB > 5.0 ng mL^{-1}	20-24 h	11 (32%) vs. 19 (17%) OR 2.3, 95%CI 1.0-5.5	Study underpowered for clinical consequences Adjudication endpoints unblinded

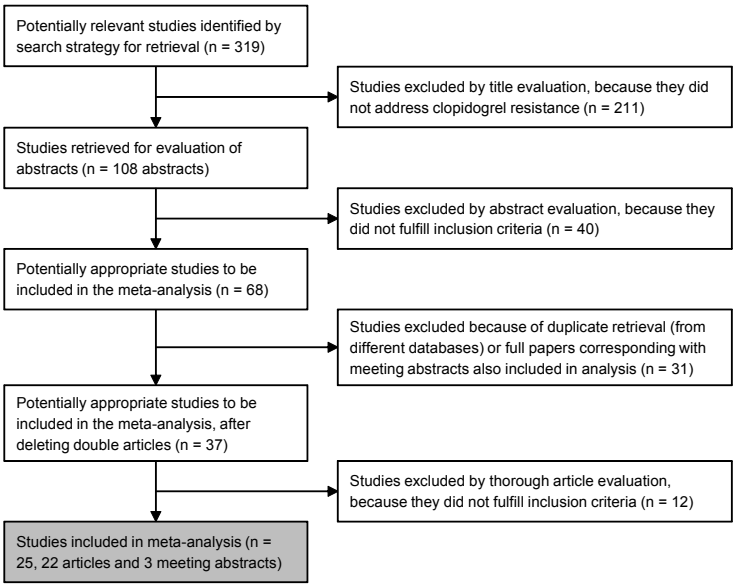

Figure 1 - Flow-chart of study selection.

Several study covariates contributed to this heterogeneity. First, a higher prevalence of non-responsiveness was observed when determined within 24 hours after loading (36%, 95%CI 28 to 44%) compared with measurements between 24 and 48 hours (13%, 95%CI 5 to 21%), two and seven days (10%, 95%CI 2 to 18%) or later (0%, 95%CI 0 to 7%). When only studies using a 600-mg clopidogrel loading dose were analyzed, there were no differences in prevalence over time.

The maintenance dose of clopidogrel was 75 mg in all studies. The used loading dose was 300 mg in all but eight studies, which used 600 mg.[14,16,30,33-35,37,39] A lower mean adjusted prevalence of non-responsiveness was found in studies using 600 mg (7%, 95%CI 0 to 15%) compared with 300 mg (22%, 95%CI 15 to 29%). In a linear regression model for prevalence of clopidogrel non-responsiveness with both time between clopidogrel loading and determination and loading dose of clopidogrel as covariates, both variables were independently inversely correlated to prevalence of non-responsiveness (*P*-values <0.001).

The methods used to evaluate the response to clopidogrel and concomitant doses of aspirin did not influence the prevalence of clopidogrel non-responsiveness.

Clinical consequences

Eight studies reported proportions of resistant and non-resistant patients undergoing PCI reaching cardiovascular end points and were eligible for pooling (Figure 2).[14,25,31-34,36,38,40-42] There was significant statistical heterogeneity among these studies (I^2=62.9, χ^2=18.87, *P*=0.009). The pooled OR of all cardiovascular outcomes was 8.0 (95%CI 3.4

Table 3 - Prevalence of laboratory clopidogrel non-responsiveness

	Mean prevalence, % (95%CI)			
	Univariate, % (95%CI)		Multivariate*, % (95 CI)	
Overall	21	(17-25)	14	(7-22)
Time between clopidogrel loading and determination				
<24 hours	27	(9-44)	36	(28-44)
24-48 hours	23	(14-32)	13	(5-21)
2-7 days	21	(15-28)	10	(2-18)
>7 days	15	(1-28)	0	(0-7)
Determination				
LTA (5 µmol/L ADP)	20	(14-25)	14	(6-23)
LTA (20 µmol/L ADP)	20	(12-29)	20	(11-29)
Other methods	24	(12-35)	9	(0-24)
Clopidogrel loading dose				
300 mg	24	(20-28)	22	(15-29)
600 mg	12	(6-18)	7	(0-15)
Aspirin dose				
≤100mg	15	(8-22)	12	(0-24)
101-300mg	24	(9-39)	14	(0-29)
≥300mg	29	(20-37)	21	(4-38)
Unknown	21	(14-27)	11	(0-26)

*Adjusted for time between clopidogrel loading and determination of clopidogrel non-responsiveness, laboratory method used, loading dose of clopidogrel and concomitant dose of aspirin.
Abbreviations: ADP: adenosine diphosphate; CI: confidence interval; LTA: light transmittance aggregometry

to 19.1). Based on these results, the PPV and NPV of non-responsiveness are 34% and 92%, respectively. Among studies reporting on occurrence of stent thrombosis,[14,31,38] the pooled OR for clopidogrel non-responsiveness was 7.0 (95%CI 0.6 to 79.0, PPV 46%, NPV 93%). When studies using a composite end point of cardiovascular events were pooled,[25,32-34] an odds ratio of 12.0 (95%CI 5.9 to 24.4, PPV 33%, NPV 96%) was found. The OR of myonecrosis[36] was 2.2 (95%CI 0.9 to 5.2, PPV 31%, NPV 83%).

Three studies were not eligible for pooling, since they did not report proportions of resistant and non-resistant patients with cardiovascular events.[40-42] In all these studies, patients suffering stent thrombosis or other cardiovascular end points exhibited a higher mean value of platelet aggregation than those without events (Table 2).

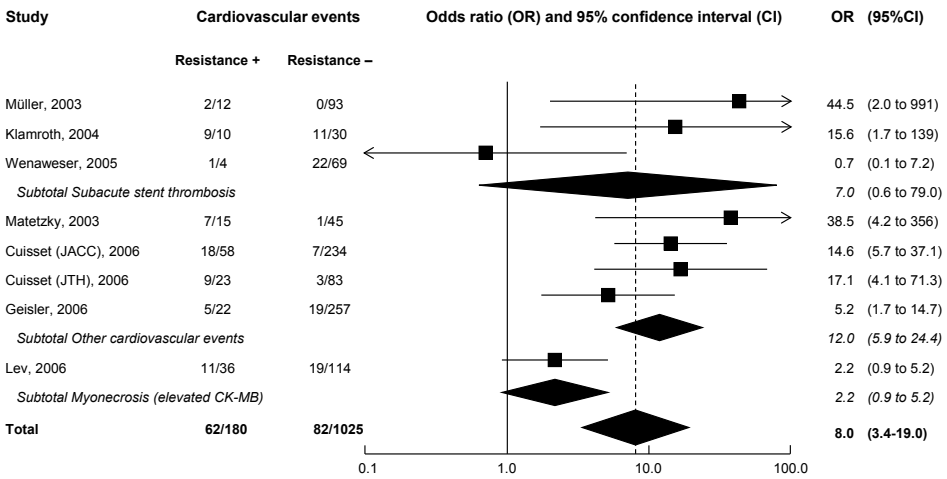

Figure 2 - Forest plot of ORs of cardiovascular outcome for clopidogrel non-responsiveness from eligible studies. Studies are grouped by outcome parameter used: (1) stent thrombosis; (2) a composite end point of clinical ischemic events, including cardiovascular death, myocardial infarction, stroke, acute coronary syndrome, revascularization and stent thrombosis; and (3) myonecrosis represented by creatine kinase-myocardial band (CK-MB) elevation after PCI.

DISCUSSION

Among studies in patients on clopidogrel to prevent cardiovascular events after PCI, our meta-analysis showed an overall prevalence of 21% of laboratory-defined clopidogrel non-responsiveness. A wide range of prevalences was found, which is partially explained by differences in time between clopidogrel loading and determination of non-responsiveness and loading dose of clopidogrel used. Our findings indicate that patients *ex vivo* labeled clopidogrel resistant have an increased risk of stent thrombosis and other cardiovascular outcomes.

Differences in reported prevalences partly depend on the moment of determining non-responsiveness. We showed that reported prevalences decreased with increasing time after clopidogrel loading. Indeed, in a large clinical trial, patients receiving a loading dose more than six hours before PCI had a favorable outcome compared with those loaded within six hours.[10] On the contrary, we found no differences over time when only studies using a 600-mg loading dose were analyzed, indicating a faster onset of action when using 600 mg. Similar to our findings, the CLEAR PLATELETS study showed that the peak inhibitory effect of clopidogrel after a 600 mg loading dose occurred at 8 hours compared with 18 to 24 hours after a 300-mg loading dose.[43] One study suggests that the full antiplatelet effect was already achieved 2 hours after using 600 mg.[44]

This meta-analysis supports the hypothesis that use of a 600 mg clopidogrel loading dose leads to a lower prevalence of clopidogrel non-responsiveness compared with 300 mg. This supports the conclusions of a study in which a stronger suppression of

platelet aggregation by 600 mg was found compared with 300 mg.[45] A randomized comparison of loading doses of 300 mg, 600 mg and 900 mg demonstrated that dosages >300 mg provide both greater reductions in platelet activation and faster onset of action compared with 300 mg.[46] Conversely, in another study it was shown that a 900-mg loading dose did not have supplemental effects beyond 600 mg because of limited clopidogrel absorption.[47] Other studies indicate that use of a 600-mg loading dose indeed leads to a lower incidence of cardiovascular events. In a trial comparing a 600 mg clopidogrel loading dose to a combination of this therapy and abciximab in 2159 patients with stable cardiovascular disease undergoing PCI, both strategies were just as effective, reinforcing the use of 600 mg clopidogrel.[48] Moreover, in a study that randomized 255 PCI patients to a clopidogrel loading dose of 300 mg and 600 mg, significantly less myocardial infarctions were found in the 600-mg group.[49]

Despite presence of statistical heterogeneity among studies, likely reflecting methodological differences, almost all included studies suggested a positive association between the risk of cardiovascular events and laboratory clopidogrel non-responsiveness. We therefore decided that it could be informative to pool these findings with a random-effects model, which partly accounts for statistical heterogeneity.[19] Our results indicate a clearly augmented overall cardiovascular risk for patients labeled laboratory clopidogrel non-responsive. The pooled risk of studies using a composite endpoint of clinical ischemic events was most obviously increased, whereas higher risks of stent thrombosis and myonecrosis were less evident, because of lack of power, even after pooling individual studies. The corresponding negative predictive values of non-responsiveness we calculated were quite high, whereas the positive predictive values were rather low. This supports the need for additional studies to examine which method of establishing the effects of clopidogrel best identifies patients at risk.

The strength of our study lies in both the systematic nature of the reviewing process and the meta-analytical method used to explain heterogeneity among prevalence data. By prespecifying inclusion criteria and a sensitive search strategy, we were able to review all retrievable studies with a minimum risk for bias. Thus, we were able to provide an extensive and, to our knowledge, complete overview on available data on both prevalence and clinical consequences of laboratory clopidogrel non-responsiveness in patients undergoing PCI, in contrary to previous reviews, which included only few studies, addressed various patient populations and did not have a systematic nature[50-53]. By pooling available studies, we were able to show a strong association between laboratory clopidogrel non-responsiveness and worsened cardiovascular outcomes.

The following potential study limitations warrant comment. First, the number of studies and patients included was relatively low. Therefore, our results have to be interpreted carefully and need to be confirmed in large prospective studies. Furthermore, as in all systematic reviews, our results may be influenced by several forms of bias. We, however, tried to minimize selection bias by using a predefined search strategy and independent selection and quality assessment by two reviewers, applying no formal language restriction and including both full-text articles and meeting abstracts. Publication and reporting bias could also have hampered our

results. However, funnel plots did not suggest this form of bias, although these forms of bias could not be completely excluded due to the limited number of studies involved.

In conclusion, our systematic review on prevalence and clinical consequences of laboratory-defined clopidogrel non-responsiveness among patients undergoing PCI indicates that in approximately one in five of them, clopidogrel non-responsiveness can be found and that this condition appears to be related to worsened clinical outcomes. Our results indicate that use of a 600-mg clopidogrel loading dose in PCI patients may result in a more rapid and stronger antiplatelet effect, which needs to be confirmed in large prospective studies. Future studies are also warranted to examine which method and time of determining clopidogrel non-responsiveness could be used in clinical practice to identify patients at the highest risk. Furthermore, there is a clear need for future studies addressing alternative strategies for these high-risk patients.

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