Clopidogrel non-responsiveness and risk of cardiovascular morbidity

An updated meta-analysis

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Summary

We performed this meta-analysis to update the clinical evidences on the relation between clopidogrel non-responsiveness and clinical outcomes in patients with coronary artery disease (CAD) who underwent percutaneous coronary intervention. An electronic literature search through MEDLINE, EMBASE, Web of Science, and the Cochrane Library and bibliographies of retrieved articles up to January, 2009 was conducted. Studies were included if they had a cohort prospective design, if they analysed clopidogrel responsiveness in CAD patients in relation to death and/or occurrence of adverse coronary events during follow-up, and if they reported an adequate statistical analysis. Fourteen studies, totalling 4,564 CAD patients followed for a time ranging from 14 days to one year, were included. The cumulative analysis reported that residual platelet reactivity despite clopidogrel treatment was significantly associated with an increased risk of death and/or thrombotic recurrences (odds ratio [OR] 5.67, 95% confidence interval [CI] 2.97 to 10.84; p<0.00001). However, four studies contributed to a consistent heterogeneity of the model and evidenced a significant risk of publication bias, so were excluded from the analysis. This exclusion, however, did not influence the overall result, by confirming the increased risk of cardiovascular recurrences for patients with a poor response to clopidogrel treatment (OR 3.58, 95%CI 2.54 to 5.05; p<0.00001). The present updated meta-analysis documents a significant association between residual platelet reactivity under clopidogrel treatment and recurrent cardiovascular events, so suggesting the relevance of ongoing interventional studies aimed at tailoring the antithrombotic therapy in CAD patients.

Keywords

Clopidogrel, antiplatelet therapy, clinical recurrences, meta-analysis

Introduction

Large clinical trials have shown that clopidogrel significantly reduces the risk of recurrent cardiovascular events in patients with coronary artery disease (CAD) (1–5). Indeed, the standard care of patients with acute coronary syndromes who underwent percutaneous coronary intervention (PCI) is, to date, the dual therapy with aspirin and clopidogrel, that has been reported to significantly decrease the occurrence of death, myocardial infarction, and stroke compared with the use of aspirin alone (1–5). Nevertheless, a wide individual variability in response to antiplatelet medications has been recently reported (6). Furthermore, an increasing amount of data on the relation between non-responsiveness to antiplatelet therapy and clinical recurrences has been reported (7, 8). Residual platelet reactivity despite aspirin treatment has been showed to be associated with an increased risk of coronary recurrences (7). Likewise, studies reporting a similar association with clopidogrel non-responsiveness have been published (9–22). Recently, Snoep et al. systematically reviewed the studies investigating the association between clopidogrel nonresponsiveness and clinical outcome, by evidencing an increased risk of clinical recurrences for those with residual platelet reactivity under clopidogrel treatment (8). However, some relevant studies on this issue from then to date have been published (19–22). Therefore, we aimed this study to update the clinical evidences on the relation between clopidogrel non-responsiveness and clinical outcomes in patients with CAD who underwent PCI.

Methods

We carried out an electronic search of MEDLINE (from 1966 to January 2009), EMBASE (from 1974 to January 2009), Science Citation Index (from 1994 to January 2009), and the Cochrane Central Register of Controlled Trials to seek for studies that investi-
gated the possible association between poor response to clopido-
grel therapy and clinical events. The search was performed using a
combined text word and MeSH search strategy of the terms. Rele-
vant keywords relating to clopidogrel therapy (”clopidogrel” or
”plavix” or ”iscover”) were used in combination with words relat-
ing to responsiveness to the therapy (”resistance” or ”resistant” or
”failure” or ”nonrespon*” or ”non-respon*” or ”low respon*” or
”low-respon*”) and to the clinical consequences (”clinical con-
sequences” or ”clinical consequence” or ”cardiovascular events” or
”adverse events” or ”recurrences” or ”major adverse cardiac events”
or ”stent thrombosis” or ”prognosis” or ”outcome”). We used no
language restrictions. Furthermore, we identified original articles
by back-referencing from reviews, and relevant studies. We as-
essed the relevance of studies by using a hierarchical approach
based on title, abstract, and the full manuscript.

Inclusion criteria

We included studies that met the following criteria: (a) cohort
prospective design, in which clinical outcomes in ”non-responder”
patients were compared with outcomes in ”responder” patients;
(b) patients were receiving clopidogrel therapy at the time of the
index event; (c) patients were classified prospectively as clopido-
grel ”non-responders” or ”responders” before the ascertainment of
any clinical outcome; (d) definition of non-responsiveness to
clopidogrel was clearly reported; (e) a measure of prospective clini-
cal outcome was used in both groups of patients; (f) relative risk,
hazard ratio, or odds ratio (OR) and their corresponding 95% con-
fidence intervals (CI) (or data to calculate them) were reported.

Outcome measures

Outcomes of interest for the current meta-analysis were major ad-
verse cardiac events (MACE), defined as any cardiovascular event
(fatal and non-fatal myocardial infarction, stroke, unstable angi-
ina), death from cardiovascular causes, ischaemic recurrences
(symptoms compatible with recurrent ischaemia needing new
hospitalisation and coronarography), stent thrombosis occurred
in CAD patients under antiplatelet therapy during follow-up.

Data collection

All data were independently extracted by two investigators (E.S.,
and R.M.) through the use of a standardised data extraction tool
and entered into separate databases. Results were compared, and
disagreements were resolved by discussion with a third investigator
(A.M.G.). The inter-observer agreement for the study selection
was 0.92. For each contributing study, the following information
was abstracted: leading author’s name, year of publication, age,
gender, number of patients, aspirin and clopidogrel dosages, du-
ration of follow-up, time of determination of response to clopido-
grel, methods used to determine the response to the therapy and
definition of clopidogrel ”resistance”, number of patients with
clinical events according to the quality of response to clopidogrel,
OR or relative risk (RR) of cardiovascular recurrences and cor-
responding 95% CI, and adjustment for potential confounders.

Statistical analysis

We pooled results from the individual studies by using Review
Manager (RevMan) software for Macintosh (version 5.0) by the
Cochrane Collaboration, and Statistical Package for Social
Sciences (SPSS) software for Windows (version 13.0). The κ sta-
tistic was used to assess agreement between reviewers for study se-
lection. The results of each study were reported as OR, RR, or di-
ichotomous frequency data. When available, we used the results of
the original studies from multivariable models with the most com-
plete adjustment for potential confounders; the confounding vari-
ables included in this analysis are shown in Table 1. We used a ran-
dom-effects model which accounts for inter-study variation and
provides a more conservative effect than the fixed model. Thus, we
calculated random-summary OR with 95% CI, by using inverse-
variance method. The potential sources of heterogeneity were as-
essed by using the Cochrane’s Q test to assess between-study dif-
fences and the $F$ statistic to quantify the proportion of inconsist-
Table 1: Characteristics of studies included in the meta-analysis.

<table>
<thead>
<tr>
<th>Source, year (reference)</th>
<th>Patients, n (age)</th>
<th>Aspirin and clopidogrel doses (mg)</th>
<th>F-UP</th>
<th>Definition of residual platelet activity</th>
<th>Time of determination</th>
<th>Outcome, n</th>
<th>Cases / responders</th>
<th>Cases / non-responders</th>
<th>OR (95%CI)</th>
<th>Adjustment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muller et al., 2003 (9)</td>
<td>105 CAD (age: ~ 60) 75M; 30F</td>
<td>Aspirin: 100 Clopidogrel: LD 600; MD 75</td>
<td>14 d</td>
<td>LTA (5, 20 μM ADP) &lt;10% reduction compared to baseline</td>
<td>4 h after LD</td>
<td>Subacute stent thrombosis (n=2)</td>
<td>0 / 93</td>
<td>2 / 12</td>
<td>44.5 (9.99–991.7)</td>
<td>None</td>
</tr>
<tr>
<td>Matetzky et al., 2004 (10)</td>
<td>60 STEMI (mean age: 58) 48M; 12F</td>
<td>Aspirin: LD 300; MD 200 Clopidogrel: LD 300; MD 75</td>
<td>6 m</td>
<td>LTA (5 μM ADP) 1st quartile of reductions compared with baseline</td>
<td>6 h after LD</td>
<td>MACE (n=8)</td>
<td>1 / 45</td>
<td>7 / 15</td>
<td>38.5 (4.15–357)</td>
<td>None</td>
</tr>
<tr>
<td>Gurbel et al., 2005 (11)</td>
<td>192 CAD (age: ~ 60) 108M; 84F</td>
<td>Aspirin: LD 325; MD: 75 Clopidogrel: LD 300 (n=75), LD 600 (n=60); MD 75</td>
<td>6 m</td>
<td>LTA (20 μM ADP) 4th quartile (&gt;72%) compared to 1–3 quartiles</td>
<td>24 h after LD</td>
<td>MACE (n=38)</td>
<td>23 / 144</td>
<td>15 / 48</td>
<td>2.39 (1.12–5.09)</td>
<td>None</td>
</tr>
<tr>
<td>Cuisset et al., 2006 (12)</td>
<td>106 ACS (mean age: 64.2) 82M; 24F</td>
<td>Aspirin: 160 Clopidogrel: LD 300; MD 75</td>
<td>1 m</td>
<td>LTA (10 μM ADP) &gt;70% vs. 1–3 quartiles</td>
<td>12 h after LD</td>
<td>MACE (n=12)</td>
<td>9 / 23</td>
<td>3 / 83</td>
<td>41.6 (4.74–364)</td>
<td>Age, gender, CV risk factors, heart rate, systolic blood pressure, treatment, CRP and P-selectin</td>
</tr>
<tr>
<td>Cuisset et al., 2006 (13)</td>
<td>292 ACS (mean age: 64.2) 222M; 70F</td>
<td>Aspirin: 160 Clopidogrel: LD 600; MD 75</td>
<td>1 m</td>
<td>LTA (10 μM ADP) &gt;70%</td>
<td>12 h after LD</td>
<td>MACE (n=18)</td>
<td>6 / 256</td>
<td>1 / 256</td>
<td>1.48 (1.05–2.08)</td>
<td>Age, gender, CV risk factors, troponin elevation, ST-segment changes, LVEF, tirofiban use</td>
</tr>
<tr>
<td>Geisler et al., 2006 (14)</td>
<td>363 CAD (mean age: 67.5) 277M; 86F</td>
<td>Aspirin: 100 Clopidogrel: LD 600; MD 75</td>
<td>3 m</td>
<td>LTA (20 μM ADP) &gt;median (14%)</td>
<td>12 h after LD</td>
<td>MACE (n=29)</td>
<td>23 / 341</td>
<td>6 / 22</td>
<td>13.7 (1.39–120.6)</td>
<td>Age, gender, diabetes, smoking habit, hypertension, LV dysfunction, prior ACS</td>
</tr>
<tr>
<td>Hochholzer et al., 2006 (15)</td>
<td>802 CAD (age: ~ 65) 627M; 175F</td>
<td>Aspirin: 100 Clopidogrel: LD 600; MD 75</td>
<td>1 m</td>
<td>LTA (5 μM ADP) &gt; median (14%)</td>
<td>At least 2 h after LD</td>
<td>MACE (n=15)</td>
<td>2 / 401</td>
<td>13 / 401</td>
<td>9.6 (2.1–44.3)</td>
<td>Age, smoking habit, hypertension, diabetess, non-left anterior descending PCI, diameter stenosis, time from clopidogrel loading</td>
</tr>
<tr>
<td>Buonamici et al., 2007 (16)</td>
<td>804 ACS (age: ~ 70) 602M; 202F</td>
<td>Aspirin: 325 Clopidogrel: LD 600; MD 75</td>
<td>6 m</td>
<td>LTA (10 μM ADP) ≥90th percentile of controls (70%)</td>
<td>12–18 h after LD</td>
<td>Stent thrombosis (n=25)</td>
<td>16 / 699</td>
<td>9 / 105</td>
<td>3.08 (1.32–7.16)</td>
<td>Age, previous MI, AMI, multivessel disease, total chronic occlusion, total stent length, LVEF, bifurcation lesion</td>
</tr>
</tbody>
</table>
ency across the study results. Sensitivity analyses were performed in order to determine the source of heterogeneity, according to the main characteristics of the study (loading dose of clopidogrel: 300 – 600 mg; duration of follow-up: <6 months – ≥6 months; method used to determine clopidogrel non-responsiveness: LTA – VerifyNow; adjustment for potential confounders: yes – no). Publication bias was assessed using a funnel plot of effect size against standard error.

Results

Database searches yielded 326 references. Exclusion of irrelevant references by title evaluation left 109 papers. After abstract evaluation additional 70 references were excluded. Moreover, after a detailed review other 25 articles were excluded because they did not fulfil our inclusion criteria. At the end of our review process 14 studies were included in the final analysis for a total of 4,564 patients (Fig. 1). Characteristics of the included studies are presented in Table 1. Five studies included CAD patients (stable angina, chronic CAD) (9, 11, 14, 15, 21) while the remaining com-
prised only acute coronary syndrome patients (10, 12, 13, 16–20, 22).

The number of participants ranged from 60 to 804, with a significantly higher prevalence of males (n=3,468; 75.9%) than females (n=1,096) and a follow-up period that ranged from 14 days to one year.

All patients were treated with aspirin with a daily dose that ranged from 75 to 325 mg. Four studies included a loading dose of clopidogrel of 300 mg (10–13), 11 studies of 600 mg (9, 11, 13, 14–20, 22), while only one study reported a loading dose of 75 mg (21). As for the maintenance dose all the studies included a dose of 75 mg/day of clopidogrel.

A variety of assays were used to assess response to clopidogrel therapy. These included light transmission aggregometry (LTA) with the use of adenosine diphosphate as agonist with different concentration in most of them (n=10) (9–17,21), point-of-care

Figure 2: Overall summary estimates of odds ratios and 95% confidence intervals (CI) for major adverse cardiac events in men and women with and without clopidogrel nonresponsiveness. Squares represent the effect size; extended lines, 95% CI; diamond, total effect size.

Figure 3: Sensitivity analyses on basis of dose of clopidogrel, duration of follow-up, method of diagnosis, adjustment for potential confounders. Squares represent the effect size; extended lines, 95% confidence intervals (CI).
testing in three studies (19, 20, 22), and vasodilator-stimulated phosphoprotein (VASP) in one paper (18).

Overall, 1,205 out of the 4,564 patients (26.4%) were classified as clopidogrel non-responders and the remaining 3,359 (73.6%) as clopidogrel responders.

The OR under a random-effects model for cardiovascular recurrences associated with a poor response to clopidogrel in each study and overall is shown in Figure 2. Compared to patients showing an optimal response to antiplatelet treatment, patients with persistent platelet reactivity despite antiplatelet treatment had a significantly higher risk of death and/or ischaemic recurrences (OR 5.67, 95% CI 2.97 to 10.84; p<0.00001). However, a consistent degree of heterogeneity was found among the 14 included studies (I²=86%; p<0.00001). All the heterogeneity was explained by studies with large RR and low number of patients and by an outlier study, that, by itself, greatly contributed (40%) to the heterogeneity of the model (9, 10, 12, 18). This paper by Blindt et al. is the only study that evaluated platelet reactivity through the VASP phosphorylation (18). But excluding these studies, indeed, the heterogeneity disappeared (I²=0%; p=0.5) while the estimate of association only slightly decreased by remaining significantly associated with an increased risk of adverse clinical cardiac events (OR 3.58, 95% CI 2.54 to 5.05; p<0.00001).

Sensitivity analyses performed after stratification of the studies into the different variables showed statistically significant results, demonstrating that characteristics of the studies (loading dose of clopidogrel, duration of follow-up, method used to determine clopidogrel response, adjustment for potential confounders) did not influence the overall results of the meta-analysis (Fig. 3). However, it should be noted that a greater estimate of association for some characteristics of the studies, such as a lower loading dose of clopidogrel (300 mg), a shorter period of follow-up (<6 months), or a method for determining platelet aggregation (LTA) can be detected with respect to their counterparts.

In conclusion, to evaluate the possible presence of publication bias among the included studies we performed a funnel plot of effect size versus standard error that reported a slightly asymmetrical visual examination, which is consistent with the conclusion that there were some statistical outliers. We identified these outliers as the studies previously observed to be the causes of the heterogeneity of the model (9, 10, 12, 18). Therefore, we excluded these studies from the analysis and the funnel plot showed no visual examination of publication bias (Fig. 4).
Discussion

The present updated meta-analysis conducted on a total population of over 4,500 CAD patients who underwent a PCI showed that persistent platelet reactivity despite clopidogrel treatment confers an increased risk of recurrent adverse cardiovascular events. Indeed, patients who were classified as clopidogrel non-responders were at about a five-fold increased risk of non-fatal and fatal cardiovascular recurrences with respect to those classified as responders.

Clinical context

Clopidogrel, in combination with aspirin, is currently the standard of care for patients undergoing PCI. Clinical trials have shown that, in high-risk patients, prolonged dual antiplatelet treatment is more effective than aspirin alone in preventing MACE (1–6). However, despite the use of such therapy, a considerable number of patients continue to have recurrent thrombotic events (9–22). Previous studies have shown a significant inter-individual variability in platelet response to clopidogrel therapy in patients with CAD, as measured by ex vivo platelet function assay, with up to 25% of patients classified as non-responders, and a growing degree of evidence that links the recurrence of adverse cardiac events to a poor response to clopidogrel is available (6, 8). Indeed, numerous clinical studies have demonstrated that clopidogrel non-responsiveness is associated with higher risk of cardiovascular events, including cardiac death and stent thrombosis (9–22). In addition, clopidogrel non-responsiveness has also been associated with a higher incidence of periprocedural myocardial damage, thrombotic complications, and long-term ischaemic events in patients undergoing PCI (23, 24).

A systematic review with meta-analysis on this issue has been recently published by Snoep et al. by reporting, as we do, that laboratory clopidogrel non-responsiveness is a marker of increased risk of adverse cardiovascular outcomes in patients undergoing PCI with stenting (8). In the present meta-analysis we have updated the issue by adding some very recent studies that comprised a considerable number of patients and by including (where available) in the cumulative analysis results obtained from a multivariable model that kept into account possible confounding factors of platelet aggregation. Hence, we have increased the number of the studied patients of the overall analysis and we have likely improved the quality of the included studies. However, despite strict inclusion criteria and in keeping with the previous meta-analysis we have found a statistical heterogeneity among the included studies. This finding could be strictly linked to the various methodological and clinical differences available among the included studies. To date, there is still a degree of uncertainty on how, when, and what consider as clopidogrel responders. There is not an accepted definition, and the timing as well as the methods to be used is not yet fully established.

In order to establish the nature of such heterogeneity we have identified four studies as outliers because of the low number of patients, the very large estimates of association and the possible risk of publication bias (9, 10, 12, 18). However, after exclusion of these studies a positive association between clopidogrel non-responsive ness and an increased risk of clinical recurrences still remained, thus confirming the overall result.

Actually, the mechanisms leading to a poor response to clopidogrel have not been fully elucidated and are considered to involve both acquired and genetic factors. Clopidogrel is a pro-drug, requiring biotransformation to an active metabolite by cytochrome P450 enzymes. The genes encoding these enzymes are polymorphic, and some studies reported some single nucleotide polymorphisms in these cytochromes able to confer reduced enzymatic function (25). Very recent studies showed that in patients with acute coronary syndromes treated with clopidogrel two different variants gene encoding for cytochrome P450 were differently associated with adverse clinical outcomes (26, 27). Moreover, clinical factors such as obesity, insulin resistance, and the nature of the coronary event have been found to contribute to the variability of the clopidogrel response (6). Another possible explanation of the resistance to thienopyridines could be the underdosing rather than in the inability of the drug to depress platelet function. It has been reported, indeed, that platelets of patients who responded poorly to clopidogrel were capable of being inhibited by a more potent thienopyridine such as prasugrel (6).

According to this, results from the different sensitivity analyses, although consistent with the main result of the meta-analysis, support the hypothesis that differences in response to clopidogrel partly depend on some methodological variables such as dosage, period of administration and methods used to determine platelet aggregation. Subgroup analyses, indeed, reported a greater risk of cardiovascular recurrences for patients treated with a lower loading dose of clopidogrel (300 mg vs. 600 mg), with a lesser period of treatment (<6 months vs. >6 months) and in whom platelet aggregation was established by means of a point-of-care versus a laboratory method. This is consistent with the findings reported by some clinical trials that recently showed a stronger suppression of platelet aggregation, and a reduced incidence of cardiovascular outcomes, in patients treated with prasugrel compared to clopidogrel (6).

What is known about this topic?

- Patients with coronary artery disease and undergoing percutaneous coronary intervention are at a much greater risk of death and/or major adverse cardiac events.
- The standard care of these patients is the dual antiplatelet treatment with aspirin and clopidogrel.
- A consistent proportion of patients under antiplatelet treatment shows a clinical non-responsiveness to the therapy.

What does this study add?

- Poor responders to clopidogrel treatment are at increased risk of cardiovascular clinical recurrences.
events, with a dosage of 600 mg of clopidogrel with respect to 300 mg (28, 29).

Limitations of study

Some potential limitations can be detected in the present meta-analysis. First, despite augmented, the number of studies is relatively low, and thus our results have to be interpreted carefully. Second, a great heterogeneity across the studies is present by likely underestimateing the overall results of the meta-analysis. On the other hand, we have performed some sensitivity analyses subgrouping studies with similar characteristics and we have reported some outlying studies within the included papers. These studies were those with greater measures of association and lower number of patients. After the exclusion, however, the overall result was confirmed. Third, publication bias in some of the included studies was supposed by means of the funnel plot.

In conclusion, our updated meta-analysis on clinical consequences of clopidogrel non-responsiveness among over than 4,500 patients followed for a period ranging from two weeks to one year after a PCI indicates that poor responders to clopidogrel are at increased risk of cardiovascular clinical recurrences with respect to those with a good response to the antiplatelet therapy.

These results suggest the need for interventional studies aimed at tailoring the antithrombotic therapy in coronary heart disease patients and need to be confirmed by further large clinical trials with standardised laboratory methods and well-defined protocols to validate the clinical relevance of such response variability to clopidogrel.

References