Thienopyridines resistance and recovery of platelet function after discontinuation of thienopyridines in cardiac surgery patients

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Abstract

OBJECTIVES: Patients who undergo cardiac operations under the effects of thienopyridines have a greater risk of major postoperative bleeding, transfusions and surgical revision due to bleeding. Discontinuation of thienopyridine is suggested but an adequate recovery period following discontinuation is still under debate, with opinions ranging from 3 to 7 days. The aim of this study was to assess the rate of recovery of thienopyridine-resistant patients and the time taken for resumption of platelet function after discontinuation of thienopyridine, in the setting of patients scheduled for cardiac operations.

METHODS: This was a retrospective study, based on 344 patients screened for platelet aggregation before cardiac operations. All the patients received thienopyridines within 7 days prior to the test. Multiple electrode aggregometry adenosine diphosphate test was used to assess platelet aggregation before the operation.

RESULTS: Thienopyridine resistance rate was 28%. Patients receiving clopidogrel had a significantly higher rate (32%) of resistance, compared with those receiving ticlopidine (14%) and thienopyridine resistance was significantly associated with platelet count (P = 0.006). The time taken to recover platelet function after thienopyridine discontinuation was variable between individuals; the only factor associated with a faster recovery time was the serum bilirubin value (P = 0.002). Platelet aggregation values high enough to avoid major bleeding were reached 3 days after discontinuation (95% confidence interval: 2–4 days); however, a complete recovery of platelet function was reached only after 8 days (95% confidence interval: 7–9 days).

CONCLUSIONS: Patient-specific factors determine the effectiveness of thienopyridine treatment and platelet function recovery rate. Among these, platelet count (for thienopyridine resistance) and serum bilirubin values (for platelet function recovery rate) should be considered.

Keywords: Platelets • Bleeding • Coronary artery bypass surgery

INTRODUCTION

Anti-platelet agents are nowadays the standard-of-care for coronary patients. Thienopyridines (ticlopidine, clopidogrel and prasugrel) are usually included in the therapeutic regimen, either alone or in association with other oral anti-platelet agents.

Patients experiencing the therapeutic effects of thienopyridines and requiring a cardiac surgery operation are at risk for major postoperative bleeding, surgical re-exploration and allogeneic blood products transfusions [1–3].

The existing international guidelines suggest discontinuation of thienopyridines before cardiac operations whenever feasible. However, there is no general consensus as to the correct time frame between the last thienopyridine administration and the day of the operation. In their previous version [4], the guidelines published by the Society of Thoracic Surgeons and the Society of Cardiovascular Anaesthesiologists suggested at least 5–7 days between discontinuation and surgery; the most recent version [5] has revised this suggestion, including a statement that in some patients, even 3 days following discontinuation may be adequate and that the use of point-of-care platelet function tests may be a reasonable means of indentifying clopidogrel non-responders who do not need any discontinuation of the drug.

However, a position statement by the Canadian Cardiovascular Society [6], suggests an interval of at least 5 days between discontinuation and surgery, balancing the risks and benefits between the risk of bleeding and of acute coronary events. Finally, the Guidelines of the European Society of Cardiology (ESC) and the European Association for Cardio-Thoracic Surgery (EACTS) [7] confirm 5 days as the minimum interval after discontinuation of thienopyridines treatment for elective patients undergoing coronary surgery.

Therefore, the present situation is characterized by a heterogeneity of opinions; moreover, the suggestion of using point-of-care platelet function tests is currently backed up by little evidence in terms of reference values, due to the paucity of studies and the use of different types of point-of-care devices.

At our institution, since 2009, all patients scheduled for heart operations—and receiving thienopyridines treatment that had not been discontinued at least one week prior to the operation—are
routinely screened with a point-of-care platelet function test. The present study is a retrospective analysis of our data for this patient population, aimed (i) to assess the rate of non-responsiveness to thienopyridine in patients and the factors associated with this condition and (ii) to assess the time required to recover platelet function after their discontinuation and the factors associated with the recovery rate.

**METHODS**

This was a single-centre, retrospective analysis of prospectively collected data. The study was approved by our local ethics committee (ASL Milano 2 Melegnano) and the need for informed consent was waived.

Starting in 2009, all patients scheduled for cardiac operations were evaluated with a pre-operative multiple electrode aggregometry (MEA) test if they were receiving treatment with thienopyridines that had not ceased at least one week prior to the operation. The MEA test was performed at the point-of-care—within the operating theatre—the day before the operation or, in the case of afternoon operations, on the morning of the operation. During the study period (January 2009 to March 2012), 344 patients were tested with MEA before their operations. Prior to 2009, it was our standard practice to interrupt the thienopyridine therapy at least 5 days before the operation; however, patients may be operated on even without discontinuation of thienopyridines if considered urgent cases. Starting in 2011, the patients could be operated on even when time since discontinuation was less than 5 days, given acceptable platelet function, indicated by the MEA adenosine diphosphate (ADP) test.

In a previous study [8] we were able to demonstrate that an MEA ADP test result of <31 U was associated with major postoperative bleeding. Therefore, during the last 12 months, the operation has usually been postponed—whenever feasible—if the MEA ADP test result was below this cut-off value, and the test was repeated until the patient reached the desired value of platelet function. Conversely, the patients were accepted for surgery if the MEA ADP test result was ≥31 U.

The use of aspirin was not an exclusion criterion. Due to the fact that aspirin does not change the MEA ADP test results, no particular measurements were done in patients taking aspirin. At our institution, aspirin is not discontinued before operation.

**Data collection**

From our institutional database, we could retrieve the following data for each patient: demographics; left ventricular ejection fraction (%); recent (30 days) myocardial infarction; unstable angina; congestive heart failure; active endocarditis; chronic obstructive pulmonary disease; diabetes on medication; previous cerebrovascular accident; previous heart operation; urgent case; haematocrit (%); serum creatinine value (mg/dl); serum bilirubin value (mg/dl); platelet count (cells/µl); type of thienopyridine used (ticlopidine, clopidogrel or prasugrel) and date of last intake; result and date of the MEA ADP test; postoperative bleeding (ml/12 h) and allogeneic blood transfusions (packed red cells, fresh frozen plasma and platelet concentrates). Postoperative bleeding was measured as the volume of blood collected from the chest drains within the first 12 postoperative hours. Packed red cell transfusions were usually triggered by a haematocrit value <24%; fresh frozen plasma and platelet concentrates were used in case of bleeding and under the guidance of postoperative thromboelastography.

**Platelet function testing**

Platelet aggregation performance was assessed using Multiplate® (Dynabyte GmbH, Munich, Germany), a point-of-care multiple electrode platelet aggregation monitoring system based on whole blood impedance measurement. The method has previously been described in detail [9]. ADP testing is sensitive towards ADP receptor inhibition induced by direct ADP receptor antagonists, such as thienopyridines. Increasing electric impedance was electronically measured for 6 min and expressed as the area under the aggregation curve (AUC), plotted over time (AUC, [U]) by an integrated software package. Reference ranges indicated by the manufacturer for ADP testing were AUCs 53–122 U.

**Platelet function: definitions**

Patients were defined as thienopyridine-resistant where:

(i) platelet aggregation value was >60% of the lower limit of the normal range, in patients who received their last thienopyridine dose within 1 day prior to the test. This corresponds to an ADP test of 32 U

(ii) platelet aggregation value >70% of the lower limit of the normal range, in patients who received their last thienopyridine dose 2 days prior to the test. This corresponds to an ADP test of 37 U.

(iii) platelet aggregation value >80% of the lower limit of the normal range, in patients who received their last thienopyridine dose 3 days prior to the test. This corresponds to an ADP test of 43 U.

(iv) platelet aggregation value within the normal range (>53 U) for patients who received their last thienopyridine dose 4–5 days prior to the test.

The above definitions are arbitrary; however, there is not yet a consensus for cut-off values defining thienopyridine non-responsiveness for the various platelet function tests [10]. Studies using MEA suggested a value of 42 U for patients under full clopidogrel treatment and are therefore compatible with our definitions [11].

The recovery of platelet function after discontinuation of thienopyridines was assessed in terms of ADP test increase per day following discontinuation. This was calculated, considering the lowest and peak values and the number of days between the two tests, according to the formula:

\[
\text{Platelet function recovery} = \frac{\text{U}}{\text{day}} = \frac{\text{(Peak ADP test U} - \text{lowest ADP test U)}}{\text{no. of days between tests}}
\]

**Statistics**

Data are expressed as number with percentage, mean and standard deviation for normally distributed variables, and median with
Poisson weighting were applied. Differences between percentages were tested with a two-sided Fisher’s exact test. Differences between continuous variables were tested with a Student’s t-test for normally distributed variables and with a Mann-Whitney U-test for non-normally distributed variables.

Associations between continuous variables were tested with linear and non-linear univariate or multivariate (stepwise forward) regression analyses. Associations between continuous variables and dichotomous variables were tested with univariate or multivariate (stepwise forward) logistic regression analyses, producing odds ratios with 95% confidence intervals (CI).

When non-normally distributed variables were entered into the regression models, adequate transformations (logarithmic) or Poisson weighting were applied. A P value <0.05 was considered significant for all the tests applied. For all the statistical analyses, a computerized package (IBM SPSS Statistics 13.0, Chicago, IL, USA) was used.

**Results**

Demographics, clinical details and laboratory data relating to our patient population are depicted in Table 1. The most commonly used thienopyridine was clopidogrel (255 patients; 80%), followed by ticlopidine (59 patients; 17%) and prasugrel (10 patients; 3%). All patients were under full dose of anti-platelet agents at the time used the thienopyridine was clopidogrel (255 patients; 80%), followed by ticlopidine (59 patients; 17%) and prasugrel (10 patients; 3%). The screening for thienopyridine resistance was conducted in 332 out of the overall population of 344 patients. The remaining twelve patients received the first MEA ADP test more than 5 days after discontinuation of the drug and presented normal values of aggregometry: for those patients, it was not possible to analyse their data for possible thienopyridine resistance. According to our definitions, 94 patients (28%) were thienopyridine-resistant. Various factors were investigated for association with thienopyridine resistance. Due to the low number of patients who received prasugrel, the type of drug used was analysed as clopidogrel (264 patients) vs ticlopidine (59 patients). The only significant differences between responders and non-responders were found in the type of thienopyridine used and patients’ platelet counts (Table 2).

Platelet count and use of clopidogrel were found to be independently associated with thienopyridine resistance in a multivariate model of logistic regression. Due to the non-normal distribution of platelet count, a logarithmic transformation was applied.

**Thienopyridine resistance**

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Table 1: Demographics, clinical details and laboratory data of the patient population (n = 344)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number (%) or mean ± standard deviation or median (interquartile range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>67.6 ± 10.6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75.0 (67–82)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170 (162–174)</td>
</tr>
<tr>
<td>Gender male</td>
<td>278 (81)</td>
</tr>
<tr>
<td>Platelet count (&gt; 1000/µl)</td>
<td>195 (158–239)</td>
</tr>
<tr>
<td>Serum creatinine value (mg/dl)</td>
<td>1.0 (0.8–1.2)</td>
</tr>
<tr>
<td>Serum bilirubin value (mg/dl)</td>
<td>0.5 (0.4–0.7)</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>39 (35–41)</td>
</tr>
<tr>
<td>Left ventricular ejection fraction (%)</td>
<td>50 (45–60)</td>
</tr>
<tr>
<td>Recent myocardial infarction</td>
<td>75 (17.7)</td>
</tr>
<tr>
<td>Unstable angina</td>
<td>22 (6.4)</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>18 (5.1)</td>
</tr>
<tr>
<td>Previous cardiac surgery</td>
<td>12 (3.5)</td>
</tr>
<tr>
<td>Diabetes on medication</td>
<td>74 (21)</td>
</tr>
<tr>
<td>Chronic obstructive pulmonary disease</td>
<td>24 (7.0)</td>
</tr>
<tr>
<td>Previous cerebrovascular accident</td>
<td>7 (2.0)</td>
</tr>
<tr>
<td>Active endocarditis</td>
<td>2 (0.6)</td>
</tr>
<tr>
<td>Urgent procedure</td>
<td>32 (9.3)</td>
</tr>
<tr>
<td>Isolated coronary operation</td>
<td>220 (64)</td>
</tr>
<tr>
<td>Isolated valve operation</td>
<td>17 (5)</td>
</tr>
<tr>
<td>Combined coronary + valve operation</td>
<td>68 (20)</td>
</tr>
<tr>
<td>Others</td>
<td>39 (11)</td>
</tr>
<tr>
<td>Ticlopidine treatment</td>
<td>59 (17)</td>
</tr>
<tr>
<td>Clopidogrel treatment</td>
<td>275 (80)</td>
</tr>
<tr>
<td>Prasugrel treatment</td>
<td>10 (3)</td>
</tr>
<tr>
<td>Days after discontinuation</td>
<td></td>
</tr>
<tr>
<td>Ongoing</td>
<td>41 (12)</td>
</tr>
<tr>
<td>One</td>
<td>58 (17)</td>
</tr>
<tr>
<td>Two</td>
<td>49 (14)</td>
</tr>
<tr>
<td>Three</td>
<td>37 (11)</td>
</tr>
<tr>
<td>Four</td>
<td>40 (12)</td>
</tr>
<tr>
<td>Five</td>
<td>30 (9)</td>
</tr>
<tr>
<td>Six or more</td>
<td>89 (25)</td>
</tr>
<tr>
<td>Postoperative bleeding (ml/12 h)</td>
<td>412 (256–600)</td>
</tr>
<tr>
<td>Packed red cells transfusions</td>
<td>189 (55)</td>
</tr>
<tr>
<td>Fresh frozen plasma transfusions</td>
<td>31 (9)</td>
</tr>
<tr>
<td>Platelet concentrates transfusions</td>
<td>55 (16)</td>
</tr>
</tbody>
</table>

Platelet function recovery

Within the population of thienopyridine responders (226 patients) the ADP test results significantly increased (P = 0.001) with increasing numbers of elapsed days following discontinuation of thienopyridine (Fig. 1). This analysis includes only one value per patient, to avoid a clustering effect. According to this relationship, the elapsed period for avoiding the risk of major bleeding (>10th decile of distribution of postoperative bleeding), according to the previously validated ADP test >31 U, is 2.5 days (95% CI: 1.5–3.5 days) and the period required for a complete recovery of platelet function is 8 days (95% CI: 7–9 days).

The 226 patients identified as thienopyridine responders received additional tests if the ADP test was <31 U and the operation was postponed. Additionally, some patients screened before 2011 received more than one test, even if the first ADP test was ≥31 U. Overall, 67 patients received two MEA tests, 22 received three and 5 received four or more MEA tests. The individual analysis for the recovery of platelet function was conducted only for thienopyridine responders who had at least two MEA tests on different days (75 cases).

The mean ADP test increase was 12 U (95% CI: 9–14) per day between the lowest and the peak value. Overall, there was a great individual variability, as shown in Fig. 2.

The main pre-operative patient characteristics collected were investigated for association with the platelet function recovery time. The only significant association was found for serum
bilirubin levels, with a greater rate of recovery for higher values of bilirubin. Due to the non-normal distribution of these values, a logarithmic transformation was applied. Figure 3 shows the platelet function recovery time according to the serum bilirubin values (data presented without logarithmic transformation).

Platelet function, postoperative bleeding and transfusions

Thienopyridine-resistant patients demonstrated a non-significant \((P = 0.116)\) lower incidence of postoperative bleeding (385 ml/12 h; interquartile range 250–600 ml/12 h) vs non-resistant patients (450 ml/12 h; interquartile range 275–606 ml/12 h).

No differences were observed for packed red cells and fresh frozen plasma transfusions; conversely, platelet concentrates were transfused at a significantly \((P = 0.05)\) higher rate in non-resistant patients (19.2%) vs resistant patients (9.9%).

The last ADP test result before the operation was significantly associated \((P = 0.002)\) with postoperative bleeding, according to a logarithmic regression analysis with Poisson weighting (Fig. 4) and with the need for postoperative platelet concentrate transfusions, according to a binary logistic regression analysis \((P = 0.001)\).

Comment

The main results of our study are:

(i) Thienopyridine resistance occurred in about 28% of the patients; at a significantly higher rate in patients treated with clopidogrel, compared with ticlopidine, and in patients with high values of platelet count.
The recovery time for platelet function in thienopyridine responders is highly variable between individuals, but significantly faster in patients with elevated serum bilirubin levels.

Platelet function immediately before a cardiac surgery operation is a determinant of postoperative bleeding and requirement for platelet concentrates transfusions.

It is well known that patients treated with ticlopidine or clopidogrel may react with different levels of platelet dysfunction, due to individual genomic patterns and the fact that, being pro-drugs, thienopyridines need absorption and subsequent liver activation to become active compounds [12, 13]. Different definitions of clopidogrel resistance have been proposed [14–16]. From the clinical point of view, anti-platelet drug resistance is a high ‘on treatment’ platelet reactivity, based on pre-defined cut-off values. In two recent works [11–16], arbitrary cut-off values with different assays have been proposed for the definition of resistance: among others, platelet aggregation >46.8 U/min in response to ADP by MEA has been set as a threshold for identification of resistant patients, both to clopidogrel and prasugrel. Another study using MEA suggested a value of 42 U for patients under full clopidogrel treatment [11].

In our study, the incidence of thienopyridine resistance (28%) is consistent with that reported in many other previous studies, reporting rates between 20–40% [17, 18].

There are several mechanisms potentially responsible for hypo- or non-responsiveness to thienopyridines. Among these, poor compliance or under-dosing, increased platelet turnover, genetic factors (i.e. polymorphisms of CYP 450 system or P2Y12 receptor) affecting both pharmacokinetics and pharmacodynamics of the agent, drug-drug interactions or comorbidities [19]. Prasugrel, because of a different bio-transformation pathway, results in faster, more consistent platelet inhibition and, at the same time, because of a different bio-transformation pathway, results in significantly faster inactivation of platelets in patients with elevated serum bilirubin levels.

Within the group of thienopyridine responders, the time required for recovery of platelet function suffers from a certain degree of individual variation: however, what is still unclear from a clinical point of view is what level of platelet function we should achieve at the MEA ADP test, that is directly proportional to platelet count, may be somehow biased by a laboratory artefact. MEA has been validated in a wide range of platelet concentrations and is independent from platelet count, at least in the range 80 000–400 000 cells/µl [18]. However, we must not fail to consider that a certain degree of impedance change at the MEA test may be ascribed to a greater platelet count, rather than to improved platelet function. Given this possible bias, our results introduce the hypothesis that anti-platelet therapy should be titrated, considering the central role of platelet count and that higher doses may be needed to adequately inactivate platelets in case of thrombocytosis.

The second main topic addressed by our study deals with the time period before cardiac surgery, following discontinuation of thienopyridines. Actually, this point deals with two different and opposite risks: that of acute coronary events in cases of premature drug cessation and that of postoperative bleeding in cases of excessive residual anti-platelet effect.

A recent meta-analysis confirms that recent exposure to clopidogrel is associated with increased risk of postoperative death, re-operations for bleeding, blood loss and need for transfusions [20]. Another systematic review demonstrates that patients exposed to thienopyridines, who undergo surgical operations (both cardiac and non-cardiac), experienced higher rates of stroke, surgical revisions due to bleeding and mortality [21].

According to a recent meta-analysis, in acute coronary syndrome patients undergoing coronary artery bypass graft, rates of mortality, myocardial infarction and major adverse cardiovascular events were no different with or without recent clopidogrel exposure [22]. However, patients recently exposed to clopidogrel had a higher surgical revision rate, greater postoperative bleeding and required more allogeneic blood product transfusions [22]. Other studies could not confirm these results [23, 24].

It is reasonable to hypothesize that one of the factors introducing a bias in these studies may be the inclusion of a variable number of thienopyridine-resistant patients, who could actually be operated on, even under full anti-platelet treatment, without additional bleeding risks. In this respect, our study confirms the potentially important role of a point-of-care test to identify thienopyridine-resistant patients and avoid unnecessary delay of the operation. Overall, the existing evidence is in favour of associating patients recently exposed to thienopyridines with a greater risk of bleeding following surgery, whereas there is greater debate over the possible beneficial effects of continuing thienopyridine therapy, in terms of reduction of major cardiovascular events.

Within the group of thienopyridine responders, the time required for recovery of platelet function suffers from a certain degree of individual variation: however, what is still unclear from a clinical point of view is what level of platelet function we should achieve in patients recently exposed to thienopyridines with a greater risk of bleeding following surgery, whereas there is greater debate over the possible beneficial effects of continuing thienopyridine therapy, in terms of reduction of major cardiovascular events.
present study. However, we can identify serum bilirubin level as a new and clinically relevant factor associated with a faster platelet function recovery time.

Patients with serum bilirubin levels exceeding 1.5 mg/dl demonstrated a platelet function recovery time in the range of 20–40 U/day. This means that, even in the presence of a total blunting of platelet function, these patients may recover an acceptable value (>31 U) of platelet function after only 24–48 h.

The effects of bilirubin on platelet function and in terms of aggregability have been recognized for more than 30 years. Unconjugated bilirubin increases the negative electrophoretic mobility of platelets. This mechanism leads to platelet aggregation, even at a bilirubin concentration of 1.5 mg/dl [25].

Altogether, the evidence that the platelet function recovery time is highly individualized—and dependent on factors that are only partially measurable—strengthens the role of point-of-care tests in following the time course of platelet function recovery after discontinuation of anti-platelet agents.

As a final confirmation of the importance of MEA ADP testing and possibly other point-of-care tests, we can confirm in our series that there is a significant and clinically relevant association between the peak value of the ADP test result immediately before the operation and the postoperative bleeding rate and need for platelet concentrates transfusions.

There are some limitations in our study. The first is its retrospective nature; the second is that, due to the changing policy on deciding to postpone the operation during the study period, not all the patients received more than one ADP test or the same number of tests. This limited the number of patients analysed for platelet function recovery time. The changes in platelet function were not assessed daily, but indirectly calculated from the elapsed time between two tests. Additionally, perceptions of major bleeding and acceptable levels of platelet function to avoid it are institutionally-based, albeit already published [10]. Finally, the definition for ‘thienopyridine resistance’ in this study (as in the previous ones) is arbitrary.

Thienopyridine treatment and its discontinuation before cardiac and other major surgical operations still remains a complex topic that involves a number of factors, the majority of which are not measurable in clinical terms. In our study we were able to confirm that platelet function point-of-care tests have a valuable role to play in assessing individual’s responses to anti-platelet agents and the recovery of their platelet function following discontinuation of anti-platelet drugs.

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Conflict of interest: none declared.

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