Correlation of inhibition of platelet aggregation after clopidogrel with post discharge bleeding events: assessment by different bleeding classifications

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Aims To correlate inhibition of platelet aggregation (IPA) with bleeding events assessed by TIMI, GUSTO, and BleedScore™ scales in a large cohort of patients with coronary artery disease (CAD) and ischaemic stroke (IS) treated with chronic low-dose aspirin plus clopidogrel. Data from recent trials and registries suggest a link between increased risk of bleeding and cardiovascular mortality. However, the potential association of bleeding risk and IPA is not established. It may play a critical role for the safety of more aggressive platelet inhibition or/and individual tailoring of antiplatelet strategies.

Methods and results Secondary post hoc analyses of 5 μM ADP-induced IPA and bleeding complications assessed by TIMI, GUSTO, and BleedScore™ scales in a combined data set consisting of patients with documented CAD (n = 246) and previous IS (n = 117). Demographic characteristics differ substantially depending on the underlying vascular disease; however, IPA and bleeding risks were similar between CAD and IS. All three bleeding scales adequately captured serious haemorrhagic events, where the TIMI scale was the most exclusive, whereas BleedScore™ was the most inclusive. Over half of all patients experienced superficial event(s), most commonly occurring during two to three distinct bleeding episodes. There was no correlation between IPA and duration of antiplatelet therapy. Inhibition of platelet aggregation >50% strongly correlates with minor ($r^2 = 0.58$, $P < 0.001$; c-statistic = 0.92), but not severe ($r^2 = 0.11$, $P = 0.038$; c-statistic = 0.57), bleeding events.

Conclusion Chronic oral combination antiplatelet regimens are associated with a very high (56.5–60.7%) prevalence of superficial bleeding episodes, which are grossly underestimated in trials and registries. The role of such frequent mild complications for the overall benefit of antiplatelet therapy is entirely unknown, as is their effect on compliance. Although IPA is well suited for defining the risk of minor complications, prediction of more severe bleeding events may be challenging.

Keywords Bleeding events • Platelet aggregation • Classifications

Introduction One of the largest controversies of modern antiplatelet strategies is the uncertain relationships among the potency of antiplatelet regimens, associated platelet inhibition, and bleeding risks. Although there are some consistent, yet controversial attempts to link randomized evidence with improved vascular outcomes and aggressiveness of antiplatelet regimens,1,2 similar data for...
platelet inhibition and bleeding events are unavailable. Some data from over a decade ago suggest that inhibition of platelet aggregation (IPA) is a useful biomarker, and that IPA of 80% is optimal for the treatment with GP IIb/IIIa inhibitors. However, excess mortality and unacceptable bleeding rates eliminated the use of these agents beyond the scope of in-hospital interventions. The cornerstone paper from the OASIS group suggests that there is a strong, consistent, temporal, and dose-related association between bleeding and death. However, it is still unclear how changes in the IPA affect bleeding risks. One of the reasons why this question remains unanswered is a difficulty to apply the appropriate type of classifications used to adequately categorize bleeding. Both conventional TIMI and GUSTO bleeding scales emerged during the era of intensive development of thrombolytic agents, when intracranial haemorrhages were the most common life-threatening complications. Therefore, both classifications may not be well suitable to assess minor bleeding episodes and individualize bleeding characteristics for each particular patient subjected to antiplatelet therapy. We introduced the BleedScore, which is based on a point accumulation. Its major feature is a cumulative, incremental expression of events by adding points up to a resulting score. In order to assess the relationship between IPA and bleeding events, we analysed bleeding data assessed using three bleeding scales from a large sample of patients with coronary artery disease (CAD) and stroke. Our hypothesis was that there would be a correlation between IPA and bleeding events.

**Methods**

**Patients**

This analysis represents a cohort of patients with CAD and ischaemic stroke (IS) treated with clopidogrel and aspirin. Patients were pooled from multiple databases yielded from six cardiology and three post-stroke protocols, had their platelets tested pre- and post-clopidogrel therapy for the IPA determination, and were available for follow-up with regard to bleeding assessment. To diminish the potential selection bias, all available data were included in the analyses. The primary study protocols were approved by the various Institutional Review Boards and performed at the different outpatient clinics, or/and hospitals in the Baltimore metropolitan area. For each study, the HeartDrug Research core laboratory was responsible for the platelet function assessment. Written informed consent was obtained from all patients who were informed of the strict compliance rules and compensated for office visits and blood draws. Compliance was assessed by interviews, pill counting, and confirmed in the majority of patients (72%) by measuring clopidogrel metabolites in plasma. Two hundred and forty-six patients with documented CAD and 117 post-IS patients who had been treated with aspirin (81 mg/daily) and clopidogrel (75 mg/daily) were eligible for this post hoc analysis. Most CAD patients (68%) also received a 300–600 mg clopidogrel loading dose immediately prior to intervention, followed by 75 mg clopidogrel once daily for at least 30 days. Others were treated with clopidogrel off-label (diabetics, and those with no documented coronary disease from ARISE and PARIS-2 databases), did not undergo coronary intervention, and received no loading dose, but 75 mg/daily maintenance regimen for at least 30 days. Both coated and uncoated aspirin formulations were allowed. Patients treated with platelet GP IIb/IIIa inhibitors were excluded from the present analysis. The diagnosis of CAD was based on the angiographic evidence of at least 30% of vessel narrowing, or/and symptoms suggestive of myocardial ischaemia assessed by electrocardiogram and an exercise stress test. Seventy-eight per cent of the patients underwent percutaneous coronary intervention(s) (PCI). The diagnosis of stroke has been confirmed by imaging; large artery atherosclerosis was diagnosed by duplex studies or angiography, showing stenosis of cerebral arteries of >50% diameter reduction with a typical morphology for atherosclerotic lesions. Cerebral microangiopathy was diagnosed if neuroimaging showed ischaemic lesions of <1.5 cm and clinical symptoms in accordance with typical lacunar syndromes (pure motor stroke, pure sensory stroke, sensorimotor stroke, dysarthria clumsy hand syndrome, and ataxic hemiparesis).

Patients from the primary studies were excluded if they had a history of bleeding diathesis, drug or alcohol abuse, prothrombin time >1.5 times control, platelet count <100 000/mm³, haematocrit <25%, or creatinine >4.0 mg/dL, surgery or angioplasty for symptomatic stenosis performed within 3 months or planned for the future, known allergy to acetylsalicylic acid or clopidogrel, history of gastrointestinal or other bleeding, history of drug-induced disorders, trauma or surgery within the last 3 months, cancer, rheumatic diseases, or seizures.

**Samples**

To be included in the present data set, all patients had a baseline sample (before clopidogrel) and at least one additional sample with evaluable 5 µM ADP-induced conventional plasma aggregation after clopidogrel. In order to reflect a patient’s full response to clopidogrel, only those patients whose platelet function tests were performed at least 4 h after a 300 mg loading dose, ≥3 h after 600 mg loading, and ≥5 days in those not receiving a loading dose and maintained on 75 mg/day were included in this analysis. Platelet function was assessed at multiple time points in different patients depending on primary protocol—at baseline, 3, 4, 24 h, 5, 30, and up to 432 days after therapy. Blood samples were obtained with a 19 G needle by direct venipuncture and drawn into 7 mL Vacutainer tubes at room temperature containing 3.8% trisodium citrate. All samples were labelled with a coded number and analysed by blinded technicians. Platelet studies were performed at baseline and at pre-specified time points as noted above.

**Platelet aggregation**

The blood–citrate mixture was centrifuged at 1200 g for 2.5 min. The resulting platelet-rich plasma (PRP) was kept at room temperature for use within 1 h. The platelet count was determined in the PRP sample and adjusted to 3.5 × 10⁶/mL with homologous platelet-poor plasma. Platelets were stimulated with 5 µmol ADP (Chronolog, Havertown, PA, USA), and aggregation was assessed as previously described using a Chronolog Lumi-Aggregometer (model 560-Ca) with the AggroLink software package. Aggregation was expressed as the maximal per cent change in light transmittance from baseline, using platelet-poor plasma as a reference. Curves were analysed according to international standards.

**Definition of inhibition of platelet aggregation**

Inhibition of platelet aggregation has been calculated in all patients by dividing post-clopidogrel number over baseline number multiplied by 100 and expressed in per cent.

For example, if pre-treatment platelet aggregation was 80%, and post-clopidogrel was 40%, then IPA is 50%. If post-clopidogrel platelet
aggregation was higher than the pre-treatment platelet activity, IPA is presented as a negative number.

**Bleeding scales**

A brief description of the TIMI, GUSTO, and BleedScore™ bleeding classifications is presented in Table 1, and affiliated questionnaire is exhibited in Table 2.

The minimal BleedScore is 0. Points are accrued on an open-ended scale. Bleeding complications are monitored continuously throughout the whole duration of the protocol. Every new bleeding event will be considered and accumulated if occurred no earlier than 15 days from the similar event. A subscript-index indicating superficial, internal, or alarming (‘S’, ‘I’, or ‘A’) is given at the end of the score, reflecting each category from which points were accrued. Examples for scores: 1s, 0i, 0a; 2s, 3i, 6a; etc. For simplified statistical assessment, e.g. in large-scale trials, points can also be combined into a pooled total BleedScore (e.g. in the examples above: pooled BleedScore 1 + 0 + 0 = 1; 2 + 3 + 6 = 11; etc.).

**Bleeding events**

Only the new-onset bleeding episodes qualified. In-hospital bleeding events which were associated with procedures did not qualify. Information on the incidence of bleeding was retrieved by open-labelled consecutive analysis of the data retrieved from the telephone interviews, face-to-face meetings, or secondary in-hospital charts, including discharge diagnosis for those patients who were hospitalized. The data were collected until clopidogrel discontinuation. Considering that the life span for platelets is ~10 days, 14 days delay between the events was required to qualify for the separate bleeding event. TIMI minor bleedings were accounted when serial haemoglobin and haematocrit data were available, whereas GUSTO scale never acknowledge minor skin bleeding episodes, although some selection bias and additional filtering may exist. Trained research nurse-co-ordinators abstracted data from medical records to case-report forms at patient discharge; and an independent research nurse-co-ordinator performed continuous source-data verification to ensure accuracy.

**Statistical analysis**

Categorical data are displayed as frequencies and percentages. The χ² test was used for dichotomous analyses of categorical data. Continuous data are presented as mean ± SD for approximately normal distributed variables and as median ± inter-quartile range for non-normal distributed variables. A simple linear regression analysis was performed to identify the relation of IPA and bleeding events. A value of r² > 0.20 has been considered clinically interesting. Discriminative power of the logistic equation has been calculated by saving the predictive probabilities from the logistic regression analysis and running a receiver operating characteristic curve with the predictive probabilities, calculating the c-statistic as the area under the curve value. The c-statistic of 1.0 represents ideal discrimination of the model, when 0.5 represents no discrimination. All significance tests

### Table 1  Bleeding classifications

<table>
<thead>
<tr>
<th>Classification</th>
<th>Severity</th>
<th>Criteria</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIMI</td>
<td>Major</td>
<td>Intracranial bleeding. Overt bleeding with a decrease in haemoglobin ≥5 g/dL or decrease in haematocrit ≥15%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Minor</td>
<td>Spontaneous gross haematuria. Spontaneous haematemesis. Observed bleeding with decrease in haemoglobin ≥3 g/dL but ≤15 or ≥10% decrease in the haematocrit</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Insignificant or minimal</td>
<td>Blood loss insufficient to meet criteria listed above with the &lt;3 g/dL of haemoglobin or &lt;9% decrease in the haematocrit</td>
<td></td>
</tr>
<tr>
<td>GUSTO</td>
<td>Severe</td>
<td>Intracerebral bleeding or substantial haemodynamic compromise requiring treatment</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>Bleeding requiring transfusion, but no haemodynamic compromise</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mild</td>
<td>Other bleeding not requiring transfusion or causing haemodynamic compromise</td>
<td></td>
</tr>
<tr>
<td>BleedScore™</td>
<td>Alarming</td>
<td>Transfusion needed, intracranial, life-threatening</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Internal</td>
<td>Haematoma, epistaxis, blood loss from mouth, vagina, melena, eye bleed, haematuria, haematemesis</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Superficial</td>
<td>Easy bruising, bleeding from small cuts, petechiae, ecchymosis</td>
<td>1</td>
</tr>
</tbody>
</table>

### Table 2  TIMI/GUSTO/BleedScore™ questionnaire

<table>
<thead>
<tr>
<th>Date</th>
<th>Bleeding event</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hospitalization</td>
<td>Yes/no</td>
</tr>
<tr>
<td></td>
<td>Haemoglobin</td>
<td>___ g/dL</td>
</tr>
<tr>
<td></td>
<td>Haematocrit</td>
<td>___ %</td>
</tr>
<tr>
<td></td>
<td>Easy bruising</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Bleeding from small cuts</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Petechiae</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Ecchymosis</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>After shaving</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>After teeth brushing</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Any other superficial bleeding</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Haematuria</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Epistaxis</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Melena</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Vaginal bleed</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Eye bleed</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Haematuria</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Haematemesis</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Mouth bleeding</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Any other internal bleeding</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Intracranial bleeding</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Blood transfusion needed</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Life-threatening bleeding</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Fatal bleeding</td>
<td>6</td>
</tr>
</tbody>
</table>
are two-sided with a nominal significance level of 5%. Statistical analyses were performed using SPSS/11.5 (SPSS, Inc., Chicago, IL, USA).

Results

Demographics and clinical characteristics

Data from the nine primary studies are outlined in Table 3.

The demographics and clinical characteristics in the combined data set in patients with CAD and after IS are presented in Table 4.

Age and gender were not distributed evenly between groups. Patients with CAD were younger and more often males as shown in Table 4. There was a prevalence of African-Americans and Asians in the post-stroke group when compared with the predominantly Caucasian CAD patients. The distribution of risk factors was also different between the two cohorts, when CAD patients exhibited more frequent family history for vascular disease, smoking, and alcohol use, whereas post-stroke patients were significantly more obese. Clinical characteristics were more similar in both groups, with the exception of previous myocardial infarction and heart surgery, which were less prevalent in stroke survivors. The distribution of concomitant medications was fairly even between groups except for warfarin, which was more common in post-stroke cohort. The timing of the second sample for the clopidogrel response assessment did not differ between groups, although the range was wide and highly variable. The second post-clopidogrel sample has been collected mostly at Day 1 in CAD patients and Day 3 in post-stroke patients.

The incidence of bleeding events dependent on the applied scale is presented in Table 5.

Among the classifications, TIMI represented the most conservative scale, whereas GUSTO captured additional bleeding events and BleedScore™ was the most liberal classification including all reported haemorrhages. Both GUSTO and BleedScore™ captured one additional serious bleeding event missed by the TIMI scale since no laboratory data on haemoglobin and haematocrit were available. Importantly, when profound events such as major and alarming (TIMI), severe and moderate (GUSTO), and alarming and internal (BleedScore™) were combined, the differences between the scales do exist. However, the main difference has been found while capturing minimal, mild, or superficial haemorrhages when the majority of these events can be captured only by BleedScore™ utilisation. The distribution of the BleedScore™ from 0 to 9 in both treatment groups is presented in Table 6.

The most commonly attributed score was ‘0’, which was recorded in over one-third of all patients suggesting that over the course of chronic antiplatelet therapy, there were no bleeding episodes. The second most frequent score was ‘2’, followed by ‘3’ indicating that most patients who experienced at least one bleeding event experienced multiple episodes rather than single (score ‘1’) haemorrhagic event. The higher scores were not frequent and represent a combination of various separate bleeding events.

The distribution of massive and superficial bleeding events over time dependent on IPA is presented in Figure 1A and B, respectively.

The analysis of the data shown in Figure 1 indicates that patients after vascular events treated with oral chronic antiplatelet agents exhibit high IPA variability independently of whether bleeding occurs. There was no correlation between timing of clopidogrel intake and bleeding risks.

Correlation between the BleedScore and the IPA is presented in Figure 2.

Analysis of the data presented at Figure 2 suggests lack of correlation ($r^2 = 0.11, P = 0.038$) and no discrimination (c-statistic $= 0.57$) between IPA and severe bleeding events. In contrast, minor superficial bleeding risks strongly depend on the degree of IPA ($r^2 = 0.58, P < 0.001$) and, more importantly, can be potentially predicted with the c-statistic value of 0.92.

Discussion

This study, composed of a large mixed population base of CAD and post-IS patients, demonstrates that: (i) oral chronic antiplatelet therapy with clopidogrel and aspirin causes wide variability of IPA despite identical treatment regimens; (ii) in contrast with significant differences in the baseline clinical characteristics, response after combination antiplatelet regimens is similar between CAD and post-IS patients; (iii) majority of the patients treated with dual antiplatelet therapy experienced superficial bleeding events; (iv) among those with bleeding events, two to three multiple

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Table 3  Characteristics of primary studies combined in the index data set

<table>
<thead>
<tr>
<th>Primary study</th>
<th>Patients</th>
<th>n</th>
<th>Loading</th>
<th>IPA assessed</th>
<th>Enrolment</th>
<th>Follow-up</th>
<th>BleedScore A/I/S*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLUTO-CAD</td>
<td>CAD</td>
<td>24</td>
<td>Yes</td>
<td>6 h, Day 30</td>
<td>2003</td>
<td>22 months</td>
<td>0/3/19</td>
</tr>
<tr>
<td>INTERACT</td>
<td>CAD</td>
<td>75</td>
<td>Yes</td>
<td>4 h, 24 h</td>
<td>2002–03</td>
<td>34 months</td>
<td>1/5/44</td>
</tr>
<tr>
<td>PLUTO-Diabetes</td>
<td>CAD</td>
<td>35</td>
<td>Yes</td>
<td>Day 30</td>
<td>2003</td>
<td>19 months</td>
<td>0/1/20</td>
</tr>
<tr>
<td>ARISE</td>
<td>CAD</td>
<td>41</td>
<td>No</td>
<td>Day 30, Day 90</td>
<td>32 months</td>
<td>32 months</td>
<td>1/4/19</td>
</tr>
<tr>
<td>PARIS-2</td>
<td>CAD</td>
<td>39</td>
<td>No</td>
<td>Day 7, Day 30</td>
<td>25 months</td>
<td>25 months</td>
<td>1/3/20</td>
</tr>
<tr>
<td>VIP-2</td>
<td>CAD</td>
<td>32</td>
<td>Yes</td>
<td>4 h, 24 h, Day 30</td>
<td>9 months</td>
<td>9 months</td>
<td>0/5/17</td>
</tr>
<tr>
<td>PLUTO-Stroke</td>
<td>Stroke</td>
<td>35</td>
<td>No</td>
<td>Day 30</td>
<td>2003–04</td>
<td>26 months</td>
<td>0/4/18</td>
</tr>
<tr>
<td>AGATE-2</td>
<td>Stroke</td>
<td>40</td>
<td>No</td>
<td>Day 30, Day 90</td>
<td>14 months</td>
<td>14 months</td>
<td>0/4/22</td>
</tr>
<tr>
<td>Stroke-Diabetes</td>
<td>Stroke</td>
<td>42</td>
<td>No</td>
<td>Day 30, Day 90</td>
<td>17 months</td>
<td>17 months</td>
<td>0/3/31</td>
</tr>
</tbody>
</table>

*A/I/S—alarming/internal/superficial bleeding events, which are not mutually exclusive.
episodes over the course of antiplatelet therapy were more common than single haemorrhage; (v) there was no relation between the IPA and duration of treatment, when bleeding events occur at the similar frequency over the time course of anti-platelet therapy; (vi) there was no relation between the degree of IPA and the incidence of internal or massive bleeding events; and (vii) there was a strong positive correlation between the IPA and superficial bleeding episodes, providing potential opportunity to predict such minor haemorrhages by serial laboratory testing of residual platelet activity.

The clinical implications of these ex vivo findings are uncertain but likely to be important, based on the emerging relevance of bleeding for the outcome success and compliance to oral antiplatelet therapies for preventive purpose. Among potential clinical implications of the index data, few deserve special attention. First, most of the patients undergoing dual antiplatelet therapy develop minor superficial bleeding events, which are usually disregarded and never reported, especially in the frame of large outcome driven studies. In fact, over half of all patients experienced at least one superficial haemorrhage. It seems highly unlikely that this finding represents some new and yet unknown complication. Most likely, these events are well known, but not captured routinely due to unclear clinical significance, and lack of the appropriate scale to make them meaningful. Not surprisingly, TIMI bleeding classification initially characterizes such bleeding events as ‘insignificant’, although their role in the achieving outcome benefit and maintaining adequate compliance seems to be important. The index study indicates that over the course of antiplatelet therapy, patients experience multiple bleeding complications, rather than a single event. Based on these data, it seems that patients usually tolerate no more than two to three such unpleasant episodes before therapy discontinuation. Dose reduction or alternative regimens may be required in this particular cohort. The expected vascular benefit.

### Table 4 Combined baseline clinical characteristics of 363 patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CAD (n = 246)</th>
<th>Ischemic stroke (n = 117)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years ± SD</td>
<td>58.1 ± 10.6</td>
<td>65.3 ± 12.8</td>
</tr>
<tr>
<td>Male</td>
<td>161 (65%)</td>
<td>37 (32%)*</td>
</tr>
<tr>
<td>Caucasian</td>
<td>149 (61%)</td>
<td>40 (34%)*</td>
</tr>
<tr>
<td>African-American</td>
<td>58 (24%)</td>
<td>51 (44%)*</td>
</tr>
<tr>
<td>Hispanic</td>
<td>26 (11%)</td>
<td>10 (8%)</td>
</tr>
<tr>
<td>Asian</td>
<td>13 (4%)</td>
<td>16 (14%)*</td>
</tr>
<tr>
<td><strong>Vascular risk factors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family history</td>
<td>158 (64%)</td>
<td>50 (43%)*</td>
</tr>
<tr>
<td>Sedentary lifestyle</td>
<td>186 (76%)</td>
<td>89 (76%)</td>
</tr>
<tr>
<td>Obesity</td>
<td>69 (28%)</td>
<td>72 (62%)*</td>
</tr>
<tr>
<td>Smoking</td>
<td>160 (65%)</td>
<td>30 (26%)*</td>
</tr>
<tr>
<td>Alcohol use</td>
<td>109 (44%)</td>
<td>17 (15%)*</td>
</tr>
<tr>
<td>Hypertension</td>
<td>164 (67%)</td>
<td>95 (81%)</td>
</tr>
<tr>
<td>Hypercholesterolaemia</td>
<td>142 (58%)</td>
<td>82 (70%)</td>
</tr>
<tr>
<td>Previous AMI</td>
<td>33 (13%)</td>
<td>2 (2%)*</td>
</tr>
<tr>
<td>Previous stroke</td>
<td>8 (3%)</td>
<td>30 (26%)*</td>
</tr>
<tr>
<td>Diabetes</td>
<td>71 (29%)</td>
<td>48 (41%)</td>
</tr>
<tr>
<td>Heart failure</td>
<td>29 (12%)</td>
<td>10 (9%)</td>
</tr>
<tr>
<td>History of CABG</td>
<td>19 (8%)</td>
<td>1 (1%)*</td>
</tr>
<tr>
<td>History of PCI</td>
<td>196 (80%)</td>
<td>187 (70%)</td>
</tr>
<tr>
<td><strong>Medications</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Timing of IPA (h ± SD)*</td>
<td>15.2 ± 11.4</td>
<td>74.0 ± 12.9*</td>
</tr>
<tr>
<td>Duration of clopidogrel, months ± SD (range)</td>
<td>6.3 ± 7.6 (1–23)</td>
<td>4.9 ± 8.4 (1–17)</td>
</tr>
<tr>
<td>Aspirin 81 mg/day</td>
<td>246 (100%)</td>
<td>117 (100%)</td>
</tr>
<tr>
<td>Statins</td>
<td>219 (89%)</td>
<td>101 (89%)</td>
</tr>
<tr>
<td>Hypotensives</td>
<td>156 (63%)</td>
<td>93 (80%)*</td>
</tr>
<tr>
<td>Warfarin</td>
<td>13 (5%)</td>
<td>32 (27%)*</td>
</tr>
<tr>
<td>Diuretics</td>
<td>23 (9%)</td>
<td>8 (7%)</td>
</tr>
</tbody>
</table>

*Time between the baseline sample (before initiation of clopidogrel therapy) and second evaluable platelet test.

*P < 0.05.
from oral antiplatelet therapy is remote, and there is no immediate improvement in clinical characteristics as seen after treatment of hypertension, or even better laboratory parameters such as changes in lipid profile after statins; these annoying bleeding events during routine tasks such as tooth brushing or shaving cause great confusion and create an illusion that antiplatelet therapy is harmful and can be discontinued.\textsuperscript{15} More disconcertingly, the patients will withdraw from both antiplatelet medications including aspirin, remaining totally unprotected from rebound platelet activation and developing secondary vascular events. The challenge is how to manage the patients after even minor bleeding episode and still on clopidogrel with regard to the discontinuation or reduction of antiplatelet therapy. This critical practical issue definitely deserve randomized outcome-driven study to determine the proper strategy.

Other important clinical implication of the index study confirms wide variability of the platelet response after clopidogrel. The bleeding complications may occur any time during the course of combination antiplatelet therapy, but the predictive value of the IPA depends heavily on the type of bleeding event. In fact, small frequent superficial haemorrhages occur predominantly when IPA is high, usually >30%. In contrast, more severe internal bleeding episodes are happening at seemingly random level of platelet inhibition, suggesting that although superficial bleeds are directly associated with platelet inhibition, the pathogenesis of larger more severe bleeding events is more complex, which may be triggered by antiplatelet therapy, but requires additional confounding haemostatic mechanisms to get involved. The fact that we can predict minor superficial but not severe internal, events by serial changes of platelet biomarkers represents the most important practical finding of our study. The BleedScore at the range 0–4 correlates well with the incidence and frequency of superficial haemorrhages, but with the BleedScore over 4 the correlation with IPA is not obvious.

Not surprisingly, the bleeding rates in our study were much lower than those reported in the major clinical trials\textsuperscript{1,16} and registries,\textsuperscript{17–19} because we deliberately did not capture frequent in-hospital events to better define the impact of oral chronic maintenance antiplatelet therapy on bleeding risks.

Our data are in full agreement with the landmark study\textsuperscript{20} suggesting that bleeding assessed with clinical criteria (GUSTO scale) is capturing more events than that assessed by laboratory criteria (TIMI scale). However, in contrast with BleedScore, both conventional classifications are not well suited for monitoring minor superficial haemorrhages.

Finally, the failure of IPA to predict severe bleeding events is clearly a disappointment; however, our expectations should not be excessive, especially considering relatively small sample size and post hoc design of the index study. Indeed, superficial events such as petechiae\textsuperscript{21} and ecchymosis\textsuperscript{22} are well-established consequences of platelet dysfunction, and it makes sense that they their occurrence correlates well with the IPA. Most likely, the mechanism for development of severe, especially catastrophic, haemorrhages is much more complex, and is probably not entirely dependent on platelet inhibition. It seems unrecognized that latent genetic defects\textsuperscript{23} should be considered suspects for the reason of why certain patients experience massive bleeding events despite average, and even low platelet inhibition after antiplatelet agents.\textsuperscript{24,25} Importantly, the index data do not match well with the bleeding rates reported in the recent clopidogrel (CURE and CREDO) and prasugrel (TRITON) trials since we assessed exclusively post-discharge bleeding risks, which are much lower than the combined in-hospital and post-discharge events. Our data are in agreement with the recent study that reported a trend towards more TIMI major and minor bleedings in PCI-treated patients with low platelet activity using Muliplate device.\textsuperscript{26} Obviously, more data are needed in the future from large prospective studies using different assays for platelet function testing and trying to link a ‘high-response’ to clopidogrel with the occurrence of bleeding events.

There are some limitations worth noting in our observations. First, we present post hoc secondary analyses, so the data were
Inhibition of platelet aggregation and bleeding events

Figure 1 Changes of inhibition of platelet aggregation dependent on the duration of antiplatelet therapy in 343 patients with coronary artery disease (squares) or post-ischaemic stroke (triangles). Grey signs represent non-bleeders (BleedScore = 0) and dark signs reflect those with bleeding events. Higher BleedScores (4+) affiliated with internal haemorrhagic events are shown in (A), whereas superficial bleedings with the BleedScore 1–3 are exhibited in (B).

not collected in a prospective fashion, with a chance for bias during the patient selection. Secondly, different prospective protocols were used for the primary studies. There was a window of ≥7 years in which the index analysis was conducted along with an evolving standard protocol resulting in unavoidable discrepancies in treatment patterns and patient management. High frequency of the use of concomitant medications may have affected the platelet characteristics; however, the overall pattern of bleeding events was similar between CAD and post-stroke patients. Since the incidence of severe bleeding events was low, lack of correlation between platelet inhibition and serious bleeding should be interpreted with caution, until more prospective data with higher statistical power became available. This is especially true since we used only a single method to assess platelet reactivity, namely platelet aggregation. Other methods may find a significant association with high IPA or post-treatment platelet reactivity and bleeding events. We also cannot deny potential mental filtering for adjudicating less minor events with the GUSTO scale, although small skin bleeding episodes are usually not counted. Timing of the second blood sampling was highly variable since no standard protocol applied. It will be important in the future to correlate the bleeding events with adverse vascular outcomes, although much larger registries will be required. Finally, compliance was not confirmed by serial detection of clopidogrel metabolites in all patients.

We conclude that chronic oral combination antiplatelet regimens are associated with very high (50–60%) risk of frequent
superficial bleeding episodes, which are grossly underestimated with TIMI and GUSTO classifications. The BleedScore classification offers an alternative method for surveillance of frequent superficial bleeding episodes when monitoring antiplatelet therapy. The role of such complications in the outcome benefit of antiplatelet therapy and maintaining compliance is unknown. Although IPA is well suited for defining the risk of minor complications, prediction of more serious bleeding events may represent a challenge. Larger studies are urgently needed to clarify this issue. The pathogenesis of catastrophic haemorrhages is more complex, spreads beyond platelet inhibition, and is probably provoked by antiplatelet therapy on top of hidden genetic haemostatic defects.

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**References**

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