High on-treatment platelet reactivity (HTPR) despite clopidogrel therapy is associated with adverse cardiac events after acute myocardial infarction (AMI). Most studies to date have assessed clopidogrel response at a single time point before or after percutaneous coronary intervention (PCI). It is unclear, however, whether the HTPR phenotype is stable over time. Therefore, we aimed to examine response to clopidogrel in patients with AMI treated with PCI over a 6-month period. Patients (n = 57) with AMI treated with PCI were assessed for response to clopidogrel at 3 time points: in hospital, 30 days, and 6 months after index hospitalization. Response to clopidogrel was determined by the VerifyNow P2Y12 assay (reported as P2Y12 response units) and multiple electrode aggregometry (MEA; reported as aggregation units). HTPR was defined as ≥235 P2Y12 response units or ≥47 aggregation units. Patients’ mean age was 54.5 ± 10.9 years, 91% were men, 19% had diabetes, and 74% were admitted with ST-segment elevation MI. HTPR based on MEA was observed in 22.8% of patients in hospital, 26.3% at 30 days, and 17.5% at 6 months (p = NS). HTPR based on the VerifyNow assay was observed in 38.6% of patients in hospital, 28.1% at 30 days, and 33.3% at 6 months (p = NS). Individual HTPR phenotypic assignment at baseline was stable in 73.7% (based on MEA) and 70.2% (based on VerifyNow) of patients at 6-month follow-up. In conclusion, this is the first study evaluating the stability of clopidogrel response over time after AMI. Rates of HTPR to clopidogrel therapy appear to be relatively stable up to 6 months after AMI. © 2012 Elsevier Inc. All rights reserved. (Am J Cardiol 2012;110:321–325)

In acute settings of ST-segment elevation myocardial infarction (MI) and non–ST-segment elevation acute coronary syndrome, increased platelet reactivity, increased platelet turnover, and an augmented inflammatory response may be associated with a decreased response to aspirin and clopidogrel.1,2 We hypothesized that as time elapses from the acute event, platelet turnover and inflammatory response would subside and, hence, response to aspirin and clopidogrel would improve. Accordingly, our aim was to evaluate and follow up to 6 months the response to aspirin and clopidogrel in patients with acute MI (AMI).

Methods

Patients who presented with ST-segment elevation MI or non–ST-segment elevation acute coronary syndrome and were treated with percutaneous coronary intervention (PCI) at the Rabin Medical Center, Israel were eligible for participation in the study. All patients received aspirin 100 mg at time of presentation, followed by 100 mg/day. A loading dose of clopidogrel 600 mg was administered preferably before the procedure (if not, immediately after PCI), followed by 75 mg/day. Adjunctive treatment during PCI (heparin, bivalirudin, and/or glycoprotein IIb/IIIa inhibitors) was given according to the physician’s discretion and preference and was documented in all patients. Specific exclusion criteria to enrollment included any contraindications for using aspirin or clopidogrel, concurrent treatment with warfarin, severe bleeding diathesis, thrombocytopenia (<100 × 10^3 cells/mm^3), anemia (hemoglobin <10 g/dl), renal insufficiency (creatinine >2.5 mg/dl), and history of a major co-morbid illness that would prevent participation during the full duration of the study. Furthermore, any patient scheduled to discontinue clopidogrel before final follow-up at 6 months was excluded from enrollment. The study was approved by the investigational review board (Helsinki committee) of the Rabin Medical Center, and all subjects provided written informed consent.

All patients were treated from the day after PCI for ≥6 months with aspirin 100 mg/day and clopidogrel 75 mg/day. All blood samples for platelet function testing were drawn from an antecubital vein using a 19-gauge needle. Samples were taken after fasting from midnight and in a state of rest. For each sample, blood was collected in tubes containing 3.2% citrate. Tubes were filled to capacity and then gently mixed. Blood samples were processed within 1 hour of blood collection. Four blood samples were drawn over the 6-month study period: (1) 18 to 24 hours after index PCI, (b) at 3 to 4 days of hospitalization and before discharge, (c) 30 days, (d) 3 months, and (e) 6 months after discharge.

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Table 1
Baseline characteristics (n = 57)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>54.5 ± 10.9</td>
</tr>
<tr>
<td>Men</td>
<td>52 (91%)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27.5 ± 3.2</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>11 (19%)</td>
</tr>
<tr>
<td>Hypertension*</td>
<td>26 (45%)</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>33 (58%)</td>
</tr>
<tr>
<td>Current or former smoker</td>
<td>22 (39%)</td>
</tr>
<tr>
<td>Family history of coronary artery disease</td>
<td>13 (23%)</td>
</tr>
<tr>
<td>Previous myocardial infarction</td>
<td>10 (18%)</td>
</tr>
<tr>
<td>Previous coronary artery bypass graft surgery</td>
<td>4 (7%)</td>
</tr>
<tr>
<td>Previous percutaneous coronary intervention</td>
<td>9 (16%)</td>
</tr>
<tr>
<td>ST-segment elevation myocardial infarction</td>
<td>42 (74%)</td>
</tr>
<tr>
<td>Non–ST-segment elevation acute coronary syndrome</td>
<td>15 (26%)</td>
</tr>
<tr>
<td>Periprocedural eptifibatide</td>
<td>43 (75%)</td>
</tr>
</tbody>
</table>

Continuous variables are presented as mean ± SD and categorical variables as number of patients (percentage).

* Blood pressure ≥140/90 mm Hg at enrollment or use of antihypertensive medication.

† Total cholesterol ≥200 mg/dl at enrollment or use of lipid-lowering medication.

Figure 1. Rates of high on-treatment platelet reactivity in response to clopidogrel defined by multiple electrode aggregometry (MEA) and VerifyNow P2Y12 assay at 3 time points. Pairwise comparisons were performed by chi-square testing.

protein IIb/IIIa may have confounded the antiplatelet effects of aspirin and clopidogrel at the early point (24 hours after PCI). Therefore, in the present analysis, only the predischarge time point (3 to 4 days after PCI) was taken into account and included in the comparisons of HTPR rates.

All platelet function parameters assessed during the study period were found to be normally distributed by the Kolmogorov–Smirnov test. Continuous variables are presented as mean ± SD. Comparison of mean platelet function measurements across various time points was performed by 2-factor analysis of variance with repeated measurements. When the interaction between the repeated-measurements factors was significant, post hoc analysis for specific pairwise comparisons was performed by Newman–Keuls tests. Comparisons between categorical variables were performed using chi-square tests. The interaction terms between important prespecified clinical variables (ST-segment elevation MI/non–ST-segment elevation acute coronary syndrome and diabetes) and platelet function over time were found to be not significant and thus further subgroup analyses were not undertaken. Analyses were performed using SPSS 11.0 (SPSS, Inc., Chicago, Illinois) and statistical significance was set at a p value ≤0.05.
Results

Fifty-seven patients were recruited who underwent urgent or emergency PCI for AMI. Of the final study participants, 42 (73.7%) developed ST-segment elevation MI and 15 (26.3%) were admitted with non–ST-segment elevation MI. Demographic characteristics, co-morbid conditions, and procedural details for all patients are presented in Table 1. Study participants had a mean age of 54.5 <H11006>10.9</H11006> years and were predominantly men (91.2%) and overweight (mean body mass index 27.5 <H11006>3.2</H11006> kg/m<sup>2</sup>). Major co-morbid conditions included hyperlipidemia (57.9%) and hypertension (45.6%). Only 9 patients (15.8%) had previously undergone PCI.

Before presentation, 22 patients (38.6%) were taking aspirin and only 2 (3.5%) were taking clopidogrel. All 42 patients with ST-segment elevation MI received the clopidogrel loading dose before PCI. Of the 15 patients with non–ST-segment elevation MI, 11 received the clopidogrel loading dose before PCI and 4 immediately after. At discharge, all patients received a statin and an angiotensin-converting enzyme inhibitor/angiotensin receptor blocker (in addition to aspirin and clopidogrel). Most patients were also discharged on a β blocker.

Rates of in-hospital HTPR despite clopidogrel therapy were 22.8% (defined by MEA–ADP test) and 38.6% (defined by VerifyNow P2Y12 assay). These rates were not significantly different at 30 days and 6 months after index hospitalization for AMI. Figure 1 shows the time course of overall rates of HTPR defined by MEA and the VerifyNow P2Y12 assay.

Table 2 lists temporal trends of mean platelet function values over time after AMI in response to dual antiplatelet therapy. MEA stimulated by arachidonic acid at baseline (bottom of panels) and during follow-up time points (top of panels). Each point represents a single patient who crossed the cut-off value for high on-treatment platelet reactivity defined by multiple electrode aggregometry (A) and the VerifyNow P2Y12 assay (B) at 3 time points.

![Figure 2. Schematic representation of high on-treatment platelet reactivity phenotypic stability in patients who developed high on-treatment platelet reactivity at baseline (bottom of panels) and during follow-up time points (top of panels). Each point represents a single patient who crossed the cut-off value for high on-treatment platelet reactivity defined by multiple electrode aggregometry (A) and the VerifyNow P2Y12 assay (B) at 3 time points.](image-url)
values did not significantly change across study time points (Table 2).

Stability of the HTPR phenotype for clopidogrel response, defined by MEA–ADP and VerifyNow P2Y12 assay, over time for individual patients is illustrated in Figure 2. Based on data from MEA, of the 13 patients who had HTPR in hospital, 8 (61.5%) remained low responders at 30 days and 5 (38.5%) had persistently high platelet reactivity at 6 months. Of the 44 responders at baseline, 7 (15.9%) subsequently crossed the cutoff for HTPR after hospital discharge. Thus, baseline assignment of phenotype (HTPR or not HTPR) defined by MEA was stable in 42 patients (73.7%) at 6-month follow-up. Based on the VerifyNow P2Y12 assay, of the 22 patients who had HTPR at baseline, 10 (45.5%) and 12 (54.5%) maintained HTPR status at 30 days and 6 months, respectively. Of the 35 responders in hospital, 7 (20%) subsequently developed high platelet reactivity at 6 months. Thus, baseline phenotype assignment (HTPR or not HTPR) defined by VerifyNow was stable in 40 patients (70.2%) at 6-month follow-up.

**Discussion**

This is the first prospective study to systematically assess clopidogrel and aspirin response stability in patients with AMI treated with PCI. Contrary to our hypothesis, overall rates of HTPR remained stable across study time points up to 6 months after AMI. Baseline classification of phenotype for clopidogrel response (HTPR or not HTPR) determined in hospital was stable in 73.7% (based on MEA) and 70.2% (based on VerifyNow assay) of patients. Despite the stability of HTPR, a decrease in mean platelet reactivity values, based on MEA induced by ADP, was noted from 30 days to 6 months after AMI. Mean platelet reactivity parameters in response to aspirin were largely stable over the follow-up period.

The findings of the present study add to existing data from stable patients undergoing elective procedures. Jaitner et al.8 evaluated patients undergoing PCI with follow-up for 15 days and found phenotypic stability in response to clopidogrel across 3 time points in 93.5% of patients based on MEA and 68% of patients based on light-transmission aggregometry. Gurbel et al.5–7 assessed patients undergoing elective coronary stenting and found that rates of “resistance” to clopidogrel, defined by light-transmission aggregometry, decreased from 31% at 24 hours after the procedure to 15% after 30 days. Only 1 study to date has followed clopidogrel response patterns up to 6 months.8 Campo et al.9 included a large cohort of patients undergoing PCI (excluding patients with ST-segment elevation MI) and found that platelet reactivity determined by the VerifyNow assay decreased significantly from baseline to 1 month but remained stable subsequently up to 6 months. Our results may differ from previous reports for a few reasons. First, the baseline in-hospital time point included in the present analysis was taken 3 to 4 days after PCI, rather than early after the procedure. However, in the 25% of patients who did not receive epifibatide, platelet reactivity was stable from 24 hours after PCI to 3 to 4 days after PCI (data not shown). Second, our study population was composed primarily of patients with ST-segment elevation MI. Recent data have shown that patients with AMI may express unique proteins in the intracoronary circulation that contribute to heightened, persistent platelet activation.9–11 Thus, the inflammatory milieu may directly influence platelet-level responsiveness to acute therapeutic interventions well into the period after PCI. In addition, the relative stability of HTPR over time in our study suggests that underlying genetic factors that contribute to alterations in platelet reactivity or clopidogrel metabolism may play a role in determining response patterns.

Although, overall, the HTPR phenotype was relatively stable in the present study, we did observe a decrease in platelet reactivity based on MEA induced by ADP from 30 days to 6 months after AMI (a finding not noted with the VerifyNow assay). It is thus plausible that as the acute inflammatory state resolves, platelet responsiveness to long-term clopidogrel therapy may improve somewhat in the late period after MI.

Determination of HTPR phenotypic stability may be clinically important to guide potential modifications of antiplatelet therapy. Antiplatelet regimens are generally prescribed in the early postprocedure time frame and thus clinical decision-making may be based on limited platelet function data (perhaps a single in-hospital ex vivo measurement). Based on our data, in-hospital determination of HTPR may be sufficient to warrant intensified antiplatelet regimens up to 6 months after AMI. In addition, these data justify current protocols for patient stratification and assignment based on platelet reactivity determination in ongoing trials involving assays such as the VerifyNow assay and the Multiplate system.

The present study has several limitations. First, postdischarge medication adherence was not accurately established in the present study. Noncompliance with prescribed antiplatelet regimens may contribute to high platelet reactivity after AMI. Second, platelet function testing in our study included MEA and the VerifyNow assay, which are 2 established tests that correlate with thrombotic risk. We did not use other assays such as light transmittance aggregometry in determining platelet reactivity at each time point. Third, although imprecision in platelet function testing can account for some degree of the observed variation in platelet reactivity over time, the reported coefficients of variation for the 2 assays are consistently <10%.12–14 Fourth, the study included a limited number of patients. Investigations including larger numbers of unstable patients are required to help validate and generalize our findings.


