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# Impact of Platelet Reactivity on Cardiovascular Outcomes in Patients With Type 2 Diabetes Mellitus and Coronary Artery Disease

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**Objectives** This study sought to determine the prognostic implications of high platelet reactivity (HPR) assessed in type 2 diabetes mellitus (T2DM) patients while in their steady-state phase of dual antiplatelet therapy.

**Background** Type 2 diabetes mellitus patients have increased platelet reactivity compared with nondiabetic patients. Whether HPR assessed in T2DM while in their steady-state phase of dual antiplatelet therapy is associated with an increased risk of major adverse cardiovascular events (MACE) is unknown.

**Methods** Platelet function analyses, which included measures of platelet aggregation and activation, were performed in 173 T2DM patients with coronary artery disease on chronic treatment with aspirin and clopidogrel. The HPR was defined as the upper quartile of maximal platelet aggregation ($Agg_{max}$) after 20 $\mu$mol/l adenosine diphosphate stimuli. Patients were followed up for 2 years and MACE were recorded.

**Results** A total of 41 MACE occurred in 34 patients (19.7%) during the 2-year follow-up. The MACE occurred in 15.2%, 12.2%, 12.2%, and 37.7% of patients from the lowest to upper quartile, respectively ($p = 0.005$). The HPR was the strongest independent predictor of MACE (hazard ratio 3.35, 95% confidence interval [CI] 1.68 to 6.66, $p = 0.001$). Receiver-operating characteristic analysis indicated that a cutoff value of 62% $Agg_{max}$ best predicted MACE (37.8% vs. 13.2%, odds ratio 3.96, 95% CI 1.8 to 8.7, $p < 0.001$). Patients with HPR had up-regulation of multiple platelet signaling pathways ($p < 0.0001$ for all assays), indicative of a global hyperreactive platelet status.

**Conclusions** High platelet reactivity determined in T2DM patients with coronary artery disease while on chronic dual antiplatelet therapy is associated with a higher risk of long-term adverse cardiovascular events, suggesting the need for tailored antithrombotic drug regimens in these high-risk patients. (J Am Coll Cardiol 2007;50:1541–7)


High platelet reactivity (HPR) has been associated with short- to mid-term atherothrombotic complications even in patients taking combined aspirin and clopidogrel therapy (1–5). However, most studies associating HPR with adverse clinical outcomes were based on functional assessments performed in the early phases of clopidogrel treatment and/or in the context of patients undergoing percutaneous coronary intervention (1–5). A considerable number of patients present with inadequate platelet inhibition early after initiation of dual antiplatelet therapy that improves with length of treatment (6,7). The clinical implications of HPR determined during a steady-state phase of dual antiplatelet therapy warrants investigation.

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High platelet reactivity is more frequent in patients with type 2 diabetes mellitus (T2DM) compared with nondiabetic patients, even when treated with dual antiplatelet

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**Abbreviations and Acronyms**

**ADP** = adenosine diphosphate

**CAD** = coronary artery disease

**HPR** = high platelet reactivity

**MACE** = major adverse cardiovascular events

**T2DM** = type 2 diabetes mellitus

therapy (8–10). Whether HPR is associated with atherothrombotic complications in T2DM patients remains to be investigated. The goal of this study was to assess platelet function profiles selectively in T2DM patients while in their chronic steady-state phase of dual antiplatelet therapy and evaluate the long-term clinical implications of HPR. We hypothesized that HPR is associated with worse long-term clinical outcomes in T2DM patients compared with those without HPR.

## Methods

**Patient population and study design.** Type 2 diabetes mellitus patients with coronary artery disease (CAD) on chronic treatment with dual antiplatelet therapy were eligible for this study. Inclusion criteria for the study were: 1) T2DM on insulin or oral hypoglycemic medication; 2) age between 18 and 80 years; 3) history of CAD; and 4) use of dual antiplatelet therapy for 6 to 9 months in the absence of cardiovascular events during this period. Type 2 diabetes mellitus was defined according to the World Health Organization report (11). All patients were recruited from the outpatient clinic of our hospital in a consecutive manner and had previously undergone percutaneous coronary intervention. Treatment with clopidogrel (75 mg/day) had been prescribed for 12 months. Aspirin (100 mg/day) was used indefinitely.

At study entry, platelet function assessment was performed and patients were divided into 4 groups based on quartile distribution of maximal 20 $\mu$mol/l adenosine diphosphate (ADP)-induced aggregation ($Agg_{max}$). Adenosine diphosphate was chosen as a platelet agonist for defining the degree of post-treatment platelet reactivity as patients were on dual antiplatelet therapy at the time of blood sampling (1). The upper quartile of ADP-induced platelet aggregation defined HPR (5). All patients were maintained on clopidogrel for an additional 3 to 6 months after study entry and were prospectively followed up for 24 months after platelet function assessment to determine the occurrence of adverse events. A flow diagram of the study design is shown in Figure 1.

Exclusion criteria for the study were as follows: 1) impaired glucose tolerance without pharmacologic treatment, gestational diabetes, or transient hyperglycemia; 2) allergy/intolerance to aspirin or clopidogrel; 3) concomitant use of antithrombotic drugs other than aspirin and clopidogrel (oral anticoagulants, dipyridamole, ticlopidine, cilostazol) or nonsteroid anti-inflammatory drugs; 4) occurrence of an acute cardiovascular event during the interval between percutaneous coronary intervention and blood sampling; 5) platelet count $<125{,}000/mm^3$; 6) hematocrit $<25\%$; 7) creatinine levels $>2.5$ mg/dl; and 8) hepatic disease (hepatic enzymes twice the upper normal limit).

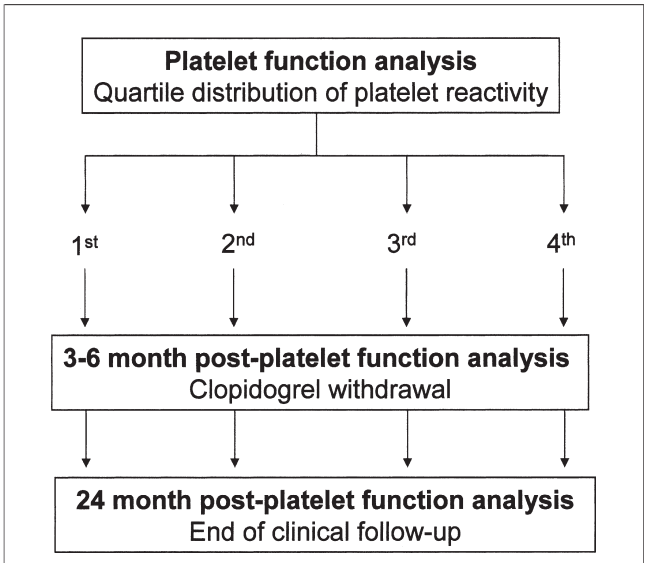


**Figure 1** Flow Diagram of the Study

This study complied with the Declaration of Helsinki and was approved by the Ethical Committee of the San Carlos University Hospital, and all patients gave their informed consent.

**Platelet function analysis.** Blood samples for platelet function assays were collected from an antecubital vein using a 21-gauge needle 2 to 4 h after antiplatelet therapy intake. The first 2 to 4 ml of blood were discarded to avoid spontaneous platelet activation. Platelet function measures included assessments of platelet aggregation and platelet activation.

Platelet aggregation was assessed using light transmittance aggregometry according to standard protocols as previously described (7–9,12). In brief, platelet aggregation was assessed using platelet-rich plasma by the turbidimetric method in a 2-channel aggregometer (ChronoLog 490 Model, ChronoLog Corp., Havertown, Pennsylvania) after 20-$\mu$mol/l ADP stimuli. The platelet-rich plasma was obtained as a supernatant after centrifugation of citrated blood at 800 rpm for 10 min. Platelet-poor plasma was obtained by a second centrifugation of the blood fraction at 2,500 rpm for 10 min. The platelet count in platelet-rich plasma was adjusted to the range of 250,000/$\mu$l by dilution with autologous plasma when platelet count was out of range. Light transmission was adjusted to 0% with platelet-rich plasma and to 100% for platelet-poor plasma for each measurement. Curves were recorded for 6 min. Aggregation was measured at peak ($Agg_{max}$) and at 5 min ($Agg_{late}$) (12). Percentage of platelet disaggregation (D) between $Agg_{max}$ and $Agg_{late}$ values was defined as: $D\ (\%) = 100 \times (1 - Agg_{late}/Agg_{max})$ (12). In addition to stimuli with ADP, other agonists nonspecific to the purinergic receptors were

used to define platelet function profiles (10,14). These included collagen (6 μg/ml), epinephrine (20 μmol/l), and thrombin receptor agonist peptide (25 μmol/l) (9,13).

Platelet activation was determined by assessing platelet surface expression of activated glycoprotein (GP) IIb/IIIa and P-selectin as previously described (7,8,14). The GP IIb/IIIa activation was assessed using PAC-1 (PAC1-FITC conjugated, Becton Dickinson, Rutherford, New Jersey) and polyclonal fluorescein isothiocyanate-conjugated rabbit anti-human fibrinogen (800 nM, Dako Diagnostics, Glostrup, Demmark) antibodies. P-selectin expression was assessed using a phycoerythrin-conjugated anti-CD62P (0.3 mg/ml, Becton Dickinson, San José, California). An Epics-XL Profile II Coulter flow cytometer (Coulter Corp. Miami, Florida) was used for the assessment. Whole blood was drawn into citrated tubes and diluted with N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES)-Tyrodes buffer to a final volume of 1:8:1 (blood to HEPES-Tyrodes to citrate) resulting in a 1:10 dilution of whole blood during sampling. Then, 50 μl of diluted whole blood was stimulated in vitro with 2-μmol/l ADP (ChronoLog) before immunolabelling. The corresponding antibody was then added and incubated for 20 min. After incubation, samples were fixed and analyzed within 2 h. Acquisition and processing data were analyzed with XL2 software (Coulter Corp.). Platelet activation was expressed as the percentage of platelets positive for antibody binding.

**Clinical end points and sample size calculation.** Patients were followed up by telephone every 6 months and via outpatient clinic visits on a yearly basis. Major adverse cardiovascular events (MACE) were defined according to definitions proposed by the American College of Cardiology and included death secondary to cardiovascular cause, ST-segment elevation myocardial infarction (STEMI), non–ST-segment elevation acute coronary syndrome (non–ST-segment elevation myocardial infarction [NSTEMI] and unstable angina [UA]), and stroke (15). Cardiovascular death was considered as any death with a demonstrable cardiovascular cause or any death that was not clearly attributable to a noncardiovascular cause. The diagnosis of STEMI was based on the evidence of new or presumably new ST-segment elevation in 2 consecutive leads and an increase in biochemical markers of myocardial necrosis. The diagnosis of NSTEMI was defined as the occurrence of ischemic symptoms, ST-segment depression and/or T-wave abnormalities, and an increase of biochemical markers of myocardial necrosis. The diagnosis of UA was defined as the occurrence of ischemic symptoms requiring hospitalization without any biochemical evidence of myocardial necrosis. Stroke was defined as the rapid onset of a new, persistent, neurologic deficit lasting at least 24 h (or resulting in death before 24 h). The treating physicians and investigators who evaluated the clinical end points were blinded to the platelet function results.

The sample size was calculated assuming a 26% incidence of MACE during the 2-year follow-up period. This estimation was based on previously published outcome data for diabetic patients (16). We hypothesized a 3-fold increase in the incidence of MACE in patients with HPR compared with patients with lower degrees of platelet reactivity. A similar magnitude of effect had been hypothesized in other platelet function studies (4,17). A sample size of at least 150 patients would be needed to fulfill these assumptions with a statistical power of 0.90 to detect the hypothesized effect size with a 2-sided p value $<0.05$.

**Statistical analysis.** Continuous variables were analyzed for a normal distribution with the Kolmogorov-Smirnov test (using p value $>0.2$ as threshold). Continuous variables following a normal distribution are expressed as mean value ± standard deviation; 1-way analysis of variance was used for comparisons across quartiles and to generate p values for trend tests. Variables not following a normal distribution are expressed as median (interquartile range); Kruskal-Wallis analysis of variance was used for comparisons across quartiles and to generate p values for trend tests. Categorical variables are expressed as frequencies and percentages. Comparisons of categorical variables were tested by the chi-square test or Fisher exact test when at least 25% of values showed an expected cell frequency below 5. Survival curves were constructed by the Kaplan-Meier method, and event-free survival among groups was compared using the log-rank test. Cox proportional hazards regression analysis was performed to identify independent correlates of the combined cardiovascular end point. The variables (demographic, clinical, and laboratory) entered in the hazards regression model were selected using stepwise regression analysis with an entry criterion of $p < 0.10$. Hazard ratio (HR) and 95% confidence intervals (CI) were calculated. Receiver-operating characteristic (ROC) analysis was performed using MedCalc version 8.2.1.0 software (Mari-

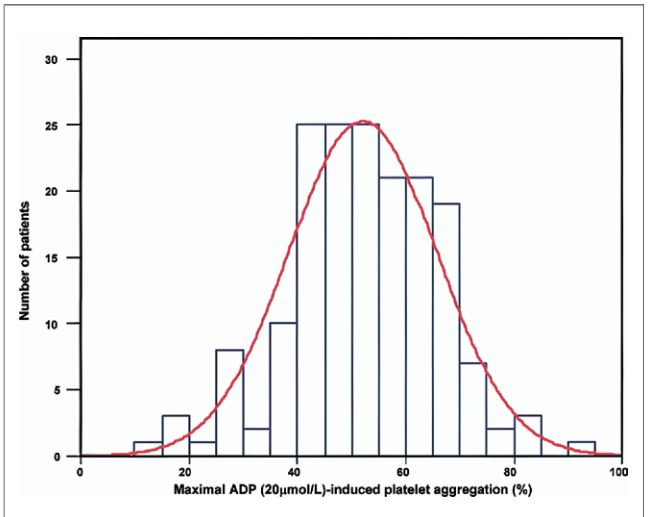


**Figure 2** Interindividual Distribution of Platelet Aggregation

Normal bell-shaped distribution of maximal adenosine diphosphate (ADP) (20 μmol/l)-induced platelet aggregation in the overall diabetic population (n = 173) assessed at study entry (6 to 9 months after initiation of dual antiplatelet therapy).

akerke, Belgium) to define sensitivity and specificity of platelet function testing. In addition, ROC analysis was used for an exploratory evaluation of the best cutoff point of $Agg_{max}$ after ADP stimuli to predict MACE in our study population; positive and negative predictive values also were derived using this cutoff value. All probability values reported are 2-sided, and a value of $p < 0.05$ was considered to be significant. Statistical analysis was performed using SPSS version 13.0 software (SPSS Inc., Chicago, Illinois).

## Results

**Baseline characteristics.** A total of 173 consecutive patients met the study inclusion criteria and were enrolled from January 2003 to February 2005. Overall, $Agg_{max}$ after ADP stimuli was 52.0 ± 14%, which was highly variable and followed a normal bell-shaped distribution (Fig. 2). Platelet reactivity quartile cut points for the 25th, 50th, and 75th percentiles of the study population were 44.0%, 52.0%, and 62.0%. The upper quartile defined HPR. The $Agg_{max}$ was 34.2 ± 8%, 47.6 ± 3%, 56.8 ± 3%, and 68.6 ± 6%, from the lowest to highest quartile, respectively ($p < 0.0001$). Baseline demographics and clinical characteristics of the study population as well as laboratory data are described in Tables 1 and 2.

**HPR and clinical outcomes.** Two-year clinical follow-up was completed in all patients. A total of 41 MACE occurred in 34 patients (19.6%) during the 2-year study follow-up period; MACE occurred in 15.2%, 12.2%, 12.2%, and 37.7% of patients from the lowest to highest quartile, respectively ($p = 0.005$). The MACE event rates were driven by UA/NSTEMI, which occurred in 9.8%, 10.9%, 12.2%, and 33.3% of patients from the lowest to highest quartile, respectively ($p = 0.007$). There were no differences in the rates of the other individual cardiovascular events. Six patients suffered more than 1 ischemic event. Four of these patients had HPR; 1 patient with 3 events had HPR. A total of 3 patients died of noncardiac causes (first quartile, 1; third quartile, 1; fourth quartile, 1) due to lung cancer, post-traumatic cerebral hematoma, and spinal fracture.

Cumulative event-free survival from MACE was significantly lower in patients with HPR compared with other groups ($p = 0.002$) (Fig. 3). Patients with MACE had higher $Agg_{max}$ (56.8 ± 13.8% vs. 50.9 ± 13.6%, $p = 0.03$) than those with an uneventful follow-up. Multivariate Cox regression analyses showed HPR (HR 3.35, 95% CI 1.68 to

**Table 1** Baseline Demographic and Clinical Characteristics of the Entire Cohort and According to Quartiles of Platelet Aggregation

| | Entire Cohort (n = 173) | 1st Quartile <44% (n = 41) | 2nd Quartile 44% to 52% (n = 46) | 3rd Quartile 52% to 62% (n = 41) | 4th Quartile >62% (n = 45) | p Value |
|---|---|---|---|---|---|---|
| Age (yrs) | 67 ± 9 | 65 ± 8 | 68 ± 10 | 67 ± 11 | 66 ± 9 | 0.77 |
| Male, n (%) | 113 (65) | 32 (78) | 29 (63) | 25 (61) | 27 (60) | 0.27 |
| Caucasian, n (%) | 173 (100) | 41 (24) | 46 (26) | 41 (24) | 45 (26) | 1.00 |
| BMI (30 kg/m$^2$) | 28.9 ± 3.9 | 28.7 ± 3.3 | 28.9 ± 4.3 | 29.2 ± 3.8 | 28.9 ± 4.3 | 0.97 |
| Risk factors/medical history, n (%) | | | | | | |
| Smoking | 23 (13) | 5 (12) | 6 (13) | 5 (12) | 7 (16) | 0.96 |
| Hypertension | 112 (65) | 26 (63) | 32 (70) | 25 (61) | 29 (64) | 0.86 |
| Dyslipidemia | 118 (68) | 3 (73) | 29 (63) | 28 (68) | 31 (69) | 0.79 |
| Obesity (BMI >30 kg/m$^2$) | 65 (38) | 13 (32) | 17 (37) | 17 (42) | 18 (40) | 0.81 |
| Insulin-treated | 54 (31) | 8 (20) | 16 (35) | 13 (32) | 17 (38) | 0.28 |
| Noninsulin treated | 119 (69) | 33 (80) | 30 (65) | 28 (68) | 28 (62) | 0.28 |
| Prior MI | 92 (53) | 19 (46) | 23 (50) | 26 (63) | 24 (53) | 0.44 |
| Prior stroke | 7 (4) | 3 (7) | 1 (2) | 2 (5) | 1 (2) | 0.57 |
| Prior CABG | 7 (4) | 5 (12) | 0 (0) | 1 (2) | 1 (2) | 0.02 |
| Symptomatic PAD | 21 (12) | 7 (17) | 6 (13) | 4 (10) | 4 (9) | 0.65 |
| Multivessel CAD | 127 (73) | 34 (83) | 31 (67) | 26 (63) | 36 (80) | 0.31 |
| Prior DES implantation | 108 (62) | 27 (66) | 27 (59) | 24 (59) | 30 (67) | 0.78 |
| NYHA functional class III or IV | 33 (19) | 3 (7) | 12 (26) | 8 (20) | 10 (22) | 0.14 |
| Chronic renal insufficiency* | 36 (21) | 2 (5) | 13 (28) | 10 (24) | 11 (24) | 0.04 |
| Medical therapy, n (%) | | | | | | |
| Beta-blockers | 120 (69) | 32 (78) | 28 (61) | 32 (78) | 28 (62) | 0.13 |
| ACE inhibitors | 94 (54) | 23 (56) | 30 (65) | 21 (51) | 20 (44) | 0.24 |
| Nitrates | 76 (44) | 17 (42) | 20 (44) | 18 (44) | 21 (47) | 0.97 |
| Lipid-lowering agents | | | | | | |
| CYP 3A4 pathway metabolized | 113 (65) | 28 (68) | 26 (57) | 25 (61) | 34 (76) | 0.24 |
| Non-CYP 3A4 pathway metabolized | 6 (4) | 1 (2) | 1 (2) | 2 (5) | 2 (4) | 0.86 |

Data are expressed as mean ± SD or number of patients (%). *Chronic renal insufficiency is defined as creatinine clearance <60 ml/min.

ACE = angiotensin-converting enzyme; BMI = body mass index; CABG = coronary artery bypass graft surgery; CAD = coronary artery disease; CYP 3A4 = hepatic cytochrome P450 3A4; DES = drug-eluting stent; MI = myocardial infarction; NYHA = New York Heart Association; PAD = peripheral arterial disease.

**Table 2** Laboratory Data of the Entire Cohort and According to Quartiles of Platelet Aggregation

| | Entire Cohort (n = 173) | 1st Quartile <44% (n = 41) | 2nd Quartile 44% to 52% (n = 46) | 3rd Quartile 52% to 62% (n = 41) | 4th Quartile >62% (n = 45) | p Value |
|---|---|---|---|---|---|---|
| Platelet count ($\times$1,000/mm$^3$) | 220 ± 47 | 207 ± 52 | 226 ± 43 | 219 ± 44 | 233 ± 54 | 0.09 |
| Hematocrit (%) | 40.1 ± 3.9 | 41.2 ± 3.9 | 40.1 ± 3.8 | 40.0 ± 3.7 | 39.0 ± 3.9 | 0.08 |
| MPV (fl) | 8.8 ± 1.1 | 8.8 ± 1.1 | 8.8 ± 1.3 | 8.9 ± 1.0 | 8.8 ± 0.9 | 0.73 |
| HbA1C (%) | 7.2 ± 1.2 | 7.0 ± 0.97 | 7.2 ± 0.92 | 7.1 ± 1.1 | 7.5 ± 1.5 | 0.09 |
| CRP (mg/dl) | 0.31 [0.18–0.68] | 0.30 [0.15–0.66] | 0.31 [0.15–0.65] | 0.33 [0.23–0.59] | 0.30 [0.18–0.79] | 0.93 |
| Creatinine (g/dl) | 1.07 ± 0.28 | 1.01 ± 0.19 | 1.09 ± 0.28 | 1.07 ± 0.26 | 1.11 ± 0.36 | 0.47 |
| Creatinine clearance (ml/min) | 75.9 ± 21.7 | 80.4 ± 16.9 | 72.4 ± 18.6 | 78.2 ± 24.3 | 73.5 ± 25.4 | 0.27 |

Data are expressed as mean ± SD or median [interquartile range].
CRP = C-reactive protein; HbA1C = glycated hemoglobin A1C; MPV = mean platelet volume.

6.66, p = 0.001), renal failure (HR 2.98, 95% CI 1.44 to 6.17, p = 0.003), and New York Heart Association functional class III to IV (HR 2.87, 95% CI 1.38 to 5.98, p = 0.005) to be independent predictors of MACE.

The ROC analysis indicated that a cutoff value of 62% $Agg_{max}$ best predicted MACE in our study population. The MACE rate was significantly higher in patients with platelet reactivity above compared with those below this cutoff value (37.7% vs. 13.3%, odds ratio [OR] 3.96, 95% CI 1.8 to 8.7, p < 0.001). This measure of platelet function showed a sensitivity and specificity of 46% and 84%, respectively. The positive and negative predictive values of 62% $Agg_{max}$ were 41% and 86%, respectively. Cumulative event-free survival from MACE using this cutoff value is shown in Figure 4. The incidence of MACE was higher in the HPR population both before (13.3% vs. 3.9%, p = 0.03) and after (24.4% vs. 9.4%, p = 0.01) clopidogrel withdrawal.

**Platelet function profile analyses.** Patients with HPR showed abnormal platelet function in all parameters assessed. Patients with HPR had enhanced $Agg_{late}$ (24.7 ± 11.3%, 36.8 ± 11.4%, 48.2 ± 7.0%, 65.1 ± 8.4% from first to fourth quartile, respectively; p < 0.0001) and diminished platelet disaggregation (30.5 ± 26.2%, 22.9 ± 23.3%, 15.4 ± 10.3%, 5.4 ± 7.0% from first to fourth quartile, respectively; p < 0.0001) compared with patients in the lower quartiles, indicative of a marked up-regulation of the $P2Y_{12}$ pathway. Platelet aggregation assessed using agonists nonspecific to purinergic receptors (collagen, epinephrine, and thrombin receptor agonist peptide) as well as markers of platelet activation (P-selectin expression and GP IIb/IIIa activation) also were all enhanced in patients with HPR

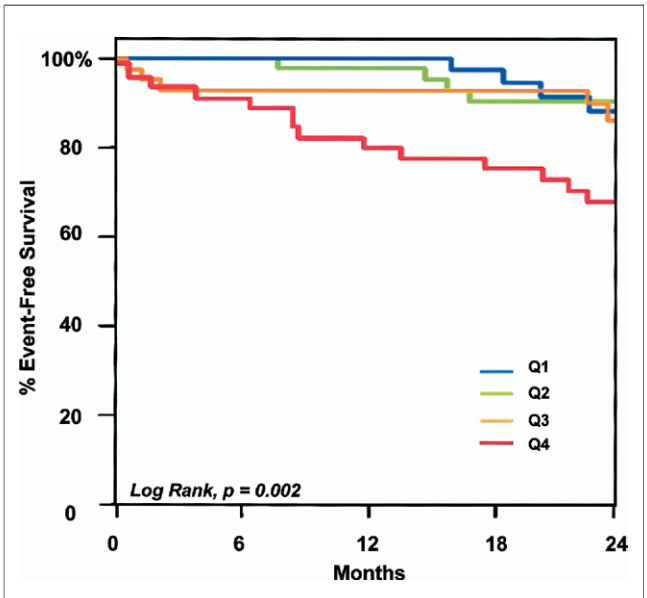


**Figure 3** Cumulative Event-Free Survival According to Quartile Distribution of Platelet Aggregation

Cumulative event-free survival from cardiovascular events according to quartile (Q) distribution of maximal adenosine diphosphate (20 μmol/l)-induced platelet aggregation.

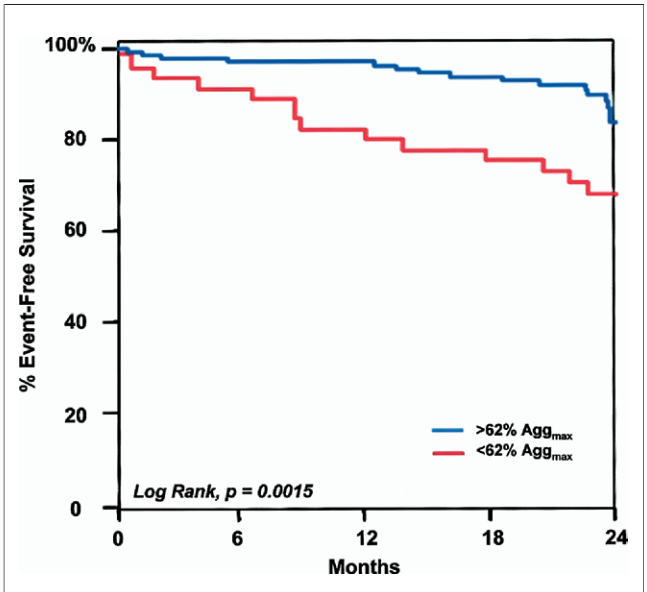


**Figure 4** Cumulative Event-Free Survival According to Optimal ROC-Defined Cutoff Value

Cumulative event-free survival from cardiovascular events according to the optimal receiver-operating characteristic (ROC)-defined cutoff value of 62% maximal adenosine diphosphate (20 μmol/l)-induced platelet aggregation ($Agg_{max}$).

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**Table 3** Platelet Function Profile Analyses

| | Entire Cohort (n = 173) | 1st Quartile <44% (n = 41) | 2nd Quartile 44% to 52% (n = 46) | 3rd Quartile 52% to 62% (n = 41) | 4th Quartile >62% (n = 45) | p Value |
|---|---|---|---|---|---|---|
| Platelet aggregation (%) | | | | | | |
| Collagen | 42.4 ± 18.5 | 25.6 ± 14.4 | 38.5 ± 17.4 | 47.1 ± 12.9 | 57.6 ± 12.2 | <0.0001 |
| Epinephrine | 31.6 ± 14.8 | 22.9 ± 13.9 | 30.7 ± 15.3 | 34.2 ± 12.7 | 38.3 ± 12.8 | <0.0001 |
| TRAP | 68.2 ± 13.6 | 57.9 ± 15.3 | 66.8 ± 12.7 | 72.4 ± 10.7 | 74.5 ± 9.4 | <0.0001 |
| Platelet activation (%) | | | | | | |
| Antifibrinogen | 30.0 ± 18.8 | 22.8 ± 20.1 | 27.5 ± 18.4 | 29.9 ± 14.9 | 39.2 ± 17.9 | <0.0001 |
| PAC-1 | 35.2 ± 19.2 | 26.9 ± 19.9 | 30.8 ± 18.9 | 37.9 ± 16.8 | 44.7 ± 16.5 | <0.0001 |
| P-selectin | 31.5 ± 18.5 | 28.6 ± 18.0 | 26.8 ± 18.6 | 28.9 ± 17.4 | 41.3 ± 16.5 | <0.0001 |

Data are expressed as mean ± SD values of percentage of platelet aggregation or percentage of positive platelets.
TRAP = thrombin receptor agonist peptide.

(Table 3). All platelet function parameters were significantly higher in patients with platelet reactivity above the ROC-determined cutoff value as well ($p < 0.0001$ for all assays).

## Discussion

The present study shows the long-term prognostic implications of HPR, defined by ADP challenge, in T2DM patients with CAD. In particular, patients with HPR determined in a steady-state phase of dual antiplatelet therapy had an over 3-fold increase in 2-year cardiovascular event rates compared with those without HPR. Our data also suggest that multiple platelet signaling pathways in addition to those mediated by the purinergic receptors are highly up-regulated in patients with HPR, indicative of a global hyperreactive platelet status.

Variability in individual response to dual antiplatelet therapy is an emerging clinical entity (1,2). To date, studies assessing the prognostic implications of inadequate platelet inhibition have been performed in patients undergoing percutaneous coronary interventions and/or in the initial phase of dual antiplatelet treatment (1–5,17–20). Studies assessing outcomes in patients undergoing percutaneous coronary interventions often are influenced by peri-interventional complications (1–5,17–20). Further, in the initial phase of dual antiplatelet therapy there are a higher proportion of patients with inadequate platelet inhibition (6,7). Also, platelet function studies in the early phases of antiplatelet therapy often include patients with acute coronary syndromes, who are more likely to have increased platelet reactivity because of an increase in plasmatic levels of von Willebrand factor (21). Overall, this enhances the likelihood of occurrence of adverse cardiovascular events. Our study provides the first evidence on the long-term prognostic value of HPR measured during a steady chronic use of dual antiplatelet therapy without the confounding association of periprocedural complications or enhanced platelet reactivity caused by an acute ischemic event.

The functional analyses performed in the present study identified a subset of patients with marked platelet hyperreactivity characterized by up-regulation of multiple platelet signaling pathways beyond purinergic receptors. This is in agreement with previous observations showing that subjects with hyperreactivity to one agonist tend to have a similar response to others, suggesting that hyperreactivity is a global characteristic of platelets (22). This may explain why many T2DM patients with elevated post-treatment platelet reactivity persist with enhanced platelet function even when treated with a high maintenance dose of clopidogrel (12). Overall, these findings corroborate previous observations suggesting that other pathways in addition to those stimulated by purinergic receptors contribute to atherothrombotic events (3). Our previous observation (23) that patients with increased platelet reactivity to nonpurinergic-specific agonists have a greater augmentation of platelet function after clopidogrel withdrawal may explain the enhanced absolute risk of developing ischemic events after clopidogrel withdrawal in patients with HPR. Whether these high-risk patients will benefit from prolonged dual antiplatelet therapy, use of more potent drugs, or antiplatelet agents inhibiting alternative targets (e.g., platelet protease-activated receptors) remains to be investigated.

Patients with T2DM are at high risk for cardiovascular events (10) and are characterized by enhanced platelet reactivity compared with nondiabetic patients (8,9). Secondary prevention measures for reduction of atherothrombotic events have been equally adopted for the overall diabetic population. However, the present report shows that T2DM patients with HPR represent an even higher-risk group within the T2DM population, suggesting the need for closer clinical monitoring in this subgroup. Although attractive, the concept of individualized antiplatelet treatment regimens based on the degree of platelet reactivity, similar to current medical management of lipid and glucose levels, remains to be tested (2,12). The major drawbacks in pursuing such a therapeutic proposition are the lack of standardization of platelet function methods as well as identification of an optimal cutoff value (1,2).

Light transmittance aggregometry is considered the gold standard methodology for platelet function analysis (1), and was used for defining the degree of post-treatment platelet reactivity in this study. Patients in the lower 3 quartiles of platelet reactivity had similar outcomes, whereas MACE

was markedly increased in the upper quartile (e.g., HPR). It seems that, until a certain threshold is reached, an increase in platelet reactivity is not paralleled by an enhanced risk of cardiovascular events. Other studies support this observation (3,4,18). We found that ~90% of patients with events had $Agg_{max}$ >40%, whereas only ~10% of patients without events had $Agg_{max}$ >62%. The ROC analyses identified an $Agg_{max}$ of 62% to best predict ischemic events in our T2DM population. This cut point had a good negative predictive value, supporting that T2DM patients with platelet reactivity below this threshold are at lower risk, with an incidence of MACE of 13.3% over a period of 2 years. Whether these findings can be used to define therapeutic goals to reduce the risk of ischemic events remains to be investigated in prospective randomized studies. In addition, the risk of bleeding with enhanced platelet inhibition must be weighed against other potential clinical benefits.

**Study limitations.** Single time point laboratory assessments represent a common limitation to most studies assessing the prognostic implications of platelet function or other biological variables (1,24,25), including the present report. In addition, PAC-1 binding and fibrinogen binding measured by flow cytometry used in this study may be influenced by native fibrinogen binding to the activated GP IIb/III receptor, and thus may not have the same value as increased P-selectin expression (26). However, the numerous laboratory assays performed in our study may have helped define more accurately the platelet function profiles of our study population.

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