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Impact of Chronic Kidney Disease on Platelet Function Profiles in Diabetes Mellitus Patients With Coronary Artery Disease Taking Dual Antiplatelet Therapy

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Objectives We sought to assess the impact of renal function on platelet reactivity in patients with diabetes mellitus (DM) and coronary artery disease on aspirin and clopidogrel therapy.

Background Diabetes mellitus is a key risk factor for chronic kidney disease (CKD). In aspirin-treated DM patients the presence of moderate/severe CKD is associated with reduced clinical efficacy of adjunctive clopidogrel therapy. Whether these findings may be attributed to differences in clopidogrel-induced effects is unknown.

Methods This was a cross-sectional observational study in which DM patients taking maintenance aspirin and clopidogrel therapy were studied. Patients were categorized into 2 groups according to the presence or absence of moderate/severe CKD. Platelet aggregation after adenosine diphosphate (ADP) and collagen stimuli were assessed with light transmittance aggregometry and defined patients with high post-treatment platelet reactivity (HPPR). Markers of platelet activation, including glycoprotein IIb/IIIa activation and P-selectin expression, were also determined using flow cytometry.

Results A total of 306 DM patients were analyzed. Patients with moderate/severe CKD ($n = 84$) had significantly higher ADP-induced ($60 \pm 13\%$ vs. $52 \pm 15\%$, $p = 0.001$) and collagen-induced ($49 \pm 20\%$ vs. $41 \pm 20\%$, $p = 0.004$) platelet aggregation compared with those without ($n = 222$). After adjustment for potential confounders, patients with moderate/severe CKD were more likely to have HPPR after ADP (adjusted odds ratio: 3.8, 95% confidence interval: 1.7 to 8.5, $p = 0.001$) and collagen (adjusted odds ratio: 2.4; 95% confidence interval: 1.1 to 5.4; $p = 0.029$) stimuli. Markers of platelet activation were significantly increased in patients with HPPR.

Conclusions In DM patients with coronary artery disease taking maintenance aspirin and clopidogrel therapy, impaired renal function is associated with reduced clopidogrel-induced antiplatelet effects and a greater prevalence of HPPR. (J Am Coll Cardiol 2010;55:1139–46) © 2010 by the American College of Cardiology Foundation

Chronic kidney disease (CKD) is estimated to affect a substantial proportion of persons aged 65 and older worldwide and is recognized as an independent predictor of myocardial infarction (MI), stroke, and all-cause mortality (1–4). Importantly, patients with CKD experience an increased risk of adverse outcomes after percutaneous coronary intervention (PCI), including stent thrombosis (5–14). Recent findings from placebo-controlled trials suggest that renal function might impact the clinical efficacy of clopidogrel. In fact, outcomes in clopidogrel-treated patients with CKD have shown to be worse compared with patients with normal renal function (15,16). Therefore, it has been hypothesized that

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Abbreviations and Acronyms

ADP = adenosine diphosphate
CAD = coronary artery disease
CI = confidence interval
CKD = chronic kidney disease
DM = diabetes mellitus
HPPR = high post-treatment platelet reactivity
MI = myocardial infarction
OR = odds ratio
PCI = percutaneous coronary intervention

CKD patients might have reduced clopidogrel-induced antiplatelet effects.

Type 2 diabetes mellitus (DM) is a key risk factor for CKD (17–19) and accounts for nearly one-half of end-stage renal disease cases in the U.S. (20). Several studies have shown DM to be associated with reduced clopidogrel response (21–25). However, whether renal function is associated with variations in clopidogrel-induced antiplatelet effects within the DM population remains unexplored. The aim of the present study was to assess the impact of renal function on pharmacodynamic profiles in aspirin-treated DM patients with coronary artery disease (CAD) taking adjunctive clopidogrel therapy.

Methods

Patient population. This is a cross-sectional observational study in which platelet function was assessed in medically treated (taking oral hypoglycemic medication and/or insulin) type 2 DM patients with stable CAD. Type 2 DM was defined according to the World Health Organization Report (26). All patients had angiographically documented CAD, because they had all previously undergone PCI. Patients were recruited at the outpatient clinic on the occasion of pre-scheduled follow-up visits at the interventional cardiology unit of the San Carlos University Hospital from April 2003 to April 2007. Patients were eligible for platelet function analyses if they were in their maintenance steady-state phase (at least 30 days) of dual antiplatelet therapy with aspirin (100 mg/day) and clopidogrel (75 mg/day). A total of 1,103 diabetic patients were screened, and 419 were identified to be taking dual antiplatelet therapy. Of the latter, 306 were eligible for the study. In the remaining 113 patients, 1 or more of the exclusion criteria indicated in the following text were identified. The rationale for assessing platelet function while patients were in their maintenance phase of treatment for at least 30 days was to overcome the variability in pharmacodynamic profiles known to occur during the initial days and weeks after initiation of therapy (27). The CKD was defined according to the National Kidney Foundation Classification as follows (28): normal renal function (creatinine clearance ≥90 ml/min), mild CKD (creatinine clearance 60 to 89 ml/min), moderate CKD (creatinine clearance 30 to 59 ml/min), and severe CKD (creatinine clearance <30 ml/min). Creatinine clearance was calculated according to the Cockroft-Gault formula (28). Due to the limited number of patients with severe CKD, these patients were combined with moderate CKD patients into 1 group (moderate/severe CKD: creatinine clearance <60 ml/min). In the present analysis, patients were first categorized into 2 groups according to the presence or absence of moderate/severe CKD. This is also in agreement with clinical studies showing that differences in outcomes in patients taking dual antiplatelet therapy were in those with advanced renal disease (10,12–16). Analyses were also performed across 3 groups (normal renal function, mild CKD, and moderate/severe CKD) in order to assess linearity in the impact of renal function on platelet function profiles.

Exclusion criteria included: known allergies to aspirin or clopidogrel; type 2 DM without pharmacological treatment; gestational diabetes; dialysis; blood dyscrasia; active bleeding or bleeding diathesis; gastrointestinal bleed within last 6 months; hemodynamic instability; acute coronary or cerebrovascular event within 3 months; any malignancy; concomitant use of other antithrombotic drugs (oral anticoagulants, dypiridamole, cilostazol, ticlopidine) or nonsteroid anti-inflammatory drugs; recent treatment (<30 days) with a glycoprotein IIb/IIIa antagonist; platelet count $<100 \times 10^6/\mu l$; hematocrit <25%; and liver disease (bilirubin level >2 mg/dl). The study complied with the Declaration of Helsinki, and all patients gave their informed written consent.

Platelet function testing. Blood sampling for platelet function analyses were collected from an antecubital vein with a 21-gauge needle 2 to 4 h after antiplatelet drug intake. The first 2 to 4 ml of blood were discarded to avoid spontaneous platelet activation, and samples were processed within 1 h. Platelet function was assessed with light transmittance aggregometry to assess platelet aggregation and flow cytometry technique to assess markers of platelet activation. Details of these assays are described elsewhere (21,23,27,29–31). In brief, platelet aggregation with light transmittance aggregometry was assessed with platelet-rich plasma by the turbidimetric method in a 2-channel aggregometer (Chrono-Log 490 Model, Chrono-Log Corp., Havertown, Pennsylvania) according to standard protocols (21,23,27,29-31). 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. Light transmission was adjusted to 0% with platelet-rich plasma and to 100% for platelet-rich plasma for each measurement. Clopidogrel-induced antiplatelet effects were assessed after challenge with adenosine diphosphate (ADP) (20 μmol/l). Nonpurinergic, mediated platelet aggregation was assessed after collagen (6 μg/ml) stimuli. Curves were recorded for 6 min, and maximal aggregation was measured. Distribution of platelet function profiles were analyzed, and high post-treatment platelet reactivity (HPPR) was defined as the upper quartile of platelet aggregation, as previously described (27,29,32). The association of renal function with HPPR to ADP ($HPPR_{ADP}$)-, collagen ($HPPR_{COLL}$)-, and both ADP and collagen ($HPPR_{ADP+COLL}$)-induced aggregation was assessed.

Markers of platelet activation were determined by assessing platelet surface expression of activated glycoprotein IIb/IIIa and P-selectin with an Epics-XL Profile II Coulter flow cytometer (Coulter Corp., Miami, Florida) as previously described (29–31). The glycoprotein IIb/IIIa activation was assessed with (PAC-1 FICT conjugated; Becton Dickinson, Rutherford, New Jersey) and polyclonal fluorescein isothiocyanate-conjugated rabbit anti-human fibrinogen (800 nmol/l, Dako Diagnostics, Glostrup, Denmark) antibodies. The P-selectin expression was assessed with a phycoerythrin-conjugated anti-CD62P (0.3 mg/ml, Becton Dickinson, San José, California). Platelet activation was expressed as the percentage of platelets positive for antibody binding.

Statistical analysis. Continuous variables were analyzed for a normal distribution with the Shapiro-Wilk test. Continuous variables following a normal distribution are presented as mean ± SD and were compared with Student unpaired *t* test. One-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) and were compared with Mann-Whitney rank-sum test. The Jonckheere-Terpstra test was used for comparisons across quartiles and to generate p values for trend tests of non-normally distributed variables. Categorical variables are presented as counts and percentages and were compared by means of the chi-square test or Fisher's exact test when at least 25% of values showed an expected cell frequency below 5. Control for potential confounders and analysis of independent correlates of HPPR were performed with a logistic regression model including age, gender, insulin-dependent diabetes, obesity, smoking, dyslipidemia, hypertension, use of calcium antagonists, angiotensin-converting enzyme inhibitors, beta-blockers, nitrates, lipophilic statins, and proton pump inhibitors as independent control variables and moderate/severe CKD as the independent study variable of interest. Odds ratio (OR) and 95% confidence interval (CI) were calculated. All probability values reported are 2-sided, and a value of $p < 0.05$ was considered significant. Statistical analysis was performed with SPSS version 15.0 software (SPSS, Inc., Chicago, Illinois).

Results

Patient population. A total of 306 type 2 DM patients were studied. Baseline demographic data, clinical characteristics, and laboratory data of patients with (n = 84) and without (n = 222) moderate/severe CKD are described in Table 1. Patients with moderate/severe CKD were more likely to be women, older, treated for multivessel CAD, and have lower body mass index and lower hematocrit (Table 1).

Platelet function profile analyses. In the overall study population, maximal ADP-induced platelet aggregation was 54 ± 15% and followed a normal bell-shaped distribution indicative of a broad variability in clopidogrel-induced antiplatelet effects (Fig. 1). Patients with moderate/severe CKD had significantly higher ADP-induced platelet aggregation compared with those without (60 ± 13% vs. 52 ± 15%, p = 0.001). Platelet reactivity quartile cut points for the 25th, 50th, and 75th percentiles of the study population were 44%, 56%, and 63%, respectively. The upper quartile identified patients with $HPPR_{ADP}$. The ADP-induced aggregation was 35 ± 9%, 51 ± 3%, 59 ± 2%, and 71 ± 7%, from the lowest to highest quartile, respectively ($p < 0.001$), and 71 ± 7% versus 48 ± 12% ($p < 0.001$) in patients with and without $HPPR_{ADP}$.

Collagen-induced aggregation was 43 ± 20% in the overall study population and was significantly higher in patients with moderate/severe CKD compared with those without (49 ± 20% vs. 41 ± 20%, p = 0.004, respectively). Quartile cut points for the 25th, 50th, and 75th percentiles were 29%, 42%, and 59%, respectively. The upper quartile identified patients with $HPPR_{COLL}$. Collagen-induced ag-

Table 1 Baseline Demographic Data, Clinical Characteristics, and Laboratory Data Stratified According to Renal Function

	Creatinine Clearance		
	≥60 ml/min (n = 222)	<60 ml/min (n = 84)	p Value
Age (yrs)	63 ± 10	72 ± 8	<0.001
Male	72.4	54.2	0.003
BMI (kg/m^2)	30 ± 4	28 ± 4	0.001
Risk factors			
Smoking	18.0	9.5	0.07
Hypertension	64.4	79.8	0.01
Dyslipidemia	68.5	70.2	0.76
Obesity (BMI >30 kg/m^2)	45.5	32.9	0.06
Insulin-treated	26.6	32.1	0.33
Medical history			
Prior MI	61.3	61.9	0.92
Prior stroke	2.3	3.6	0.69
Prior CABG	5.3	7.7	0.41
Symptomatic PAD	9.5	11.9	0.53
Multivessel CAD	61.5	80.2	0.002
Medical therapy			
Beta-blockers	74.8	64.3	0.07
ACE inhibitors	59.9	63.1	0.61
Nitrates	36.5	40.5	0.52
Ca^{2+} antagonists	25.6	32.8	0.27
Lipid-lowering agents			
CYP 3A4 pathway metabolized	65.7	70.3	0.50
Non-CYP 3A4 pathway metabolized	5.4	6.3	0.76
Proton pump inhibitors	32.5	31.3	0.85
Laboratory data			
Platelet count (1,000/mm^3)	223 ± 58	226 ± 61	0.75
Hematocrit (%)	41 ± 4	39 ± 5	<0.001
MPV (fl)	8.9 ± 1.1	8.7 ± 0.9	0.27
HbA1C (%)	7.1 ± 1.2	7.0 ± 1.4	0.91
Creatinine (g/dl)	0.9 ± 0.2	1.3 ± 0.5	<0.001

Values are mean ± SD or %.

ACE = angiotensin-converting enzyme; BMI = body mass index; CABG = coronary artery bypass graft surgery; CAD = coronary artery disease; CYP 3A4 = hepatic cytochrome P450 3A4; HbA1C = glycated hemoglobin A1C; MI = myocardial infarction; MPV = mean platelet volume; PAD = peripheral artery disease.

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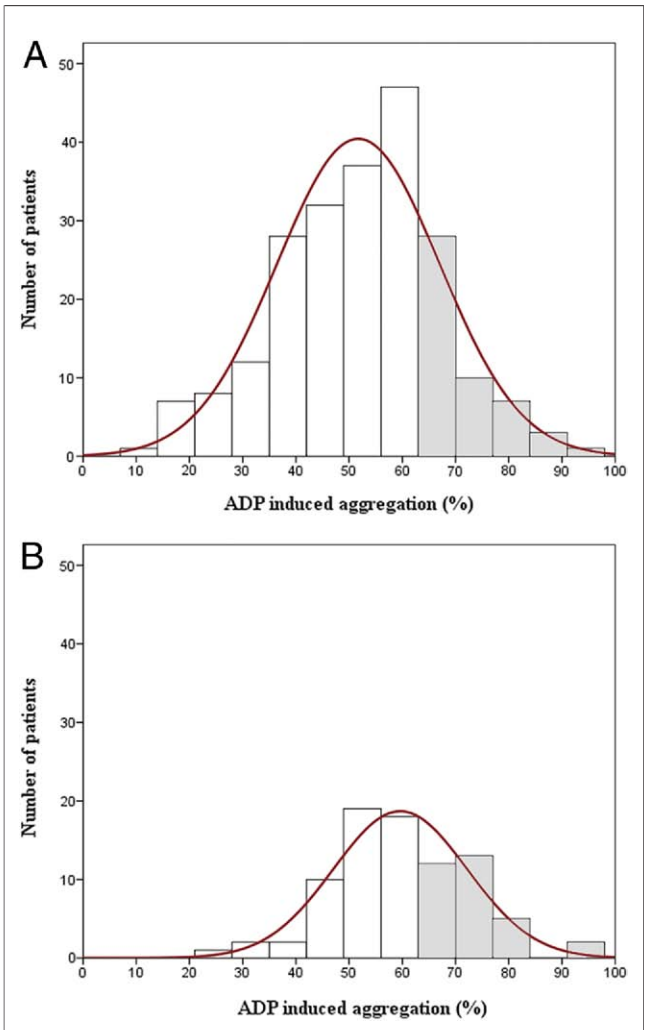

Figure 1 **Interindividual Distribution of Platelet Aggregation Stratified by Renal Function**

Normal bell-shaped distribution of maximal adenosine diphosphate (ADP) (20 μmol/l)-induced platelet aggregation in patients with normal or mildly impaired renal function **(A)** and patients with moderate or severe chronic renal failure **(B)** assessed at study entry. **Shaded bars** denote the distribution of patients with high post-treatment platelet reactivity in the study groups.

gregation was 17 ± 8%, 37 ± 4%, 51 ± 5%, and 68 ± 9% from the lowest to highest quartile, respectively ($p < 0.001$), and 68 ± 9% versus 35 ± 15% ($p < 0.001$) in patients with and without $HPPR_{COLL}$.

Collagen-induced aggregation was higher (57 ± 18% vs. 39 ± 19%, $p < 0.001$) in patients with and without $HPPR_{ADP}$. Similarly, ADP-induced aggregation was higher (65 ± 13% vs. 50 ± 14%, $p < 0.001$) in patients with and without $HPPR_{COLL}$. Among patients with $HPPR_{ADP}$, 57.9% had $HPPR_{COLL}$. Among patients with $HPPR_{COLL}$, 57.1% had $HPPR_{ADP}$. Patients with $HPPR_{ADP+COLL}$ (14.4% of the total population) had significantly enhanced ADP- (74 ± 8% vs. 51 ± 13%, $p < 0.001$) and collagen-induced (69 ± 9% vs. 39 ± 18%, $p < 0.001$) platelet aggregation. Markers of platelet activation were consistently increased in patients with $HPPR_{ADP}$, $HPPR_{COLL}$, and $HPPR_{ADP+COLL}$ (Table 2).

Platelet function across different stages of CKD. Analysis of variance demonstrated significantly lower ADP- ($p = 0.001$) and collagen-induced ($p = 0.016$) platelet aggregation in patients with normal renal function compared with those with mild CKD and those with moderate/severe CKD (Fig. 2). However, no significant differences in platelet aggregation were observed between patients with normal renal function and those with mild CKD (Fig. 2).

CKD and HPPR. Patients with moderate/severe CKD were more likely to have $HPPR_{ADP}$ than those without moderate/severe CKD (35.7% vs. 20.8%, OR: 2.1, 95% CI: 1.2 to 3.7, $p = 0.007$). After adjustment for potential confounders, moderate/severe CKD remained significantly associated with $HPPR_{ADP}$ (adjusted OR: 3.8, 95% CI: 1.7 to 8.5, $p = 0.001$). Intake of proton-pump inhibitors (adjusted OR: 2.78, 95% CI: 1.36 to 5.71, $p = 0.005$) was also significantly associated with $HPPR_{ADP}$. Similarly, higher rates of moderate/severe CKD were seen in patients with $HPPR_{COLL}$ (33.3% vs. 22.1%; OR: 1.8; 95% CI: 1.0 to 3.1; $p = 0.043$; adjusted OR: 2.4; 95% CI: 1.1 to 5.4; $p = 0.029$) and in those with $HPPR_{ADP+COLL}$ (23.8% vs. 10.8%; OR: 2.6; 95% CI: 1.3 to 5.0; $p = 0.004$; adjusted OR: 3.3; 95% CI: 1.3 to 8.4; $p = 0.01$) (Fig. 3).

Discussion

The present study demonstrates that in type 2 DM patients with CAD receiving maintenance aspirin and clopidogrel therapy, the presence of moderate/severe CKD is associated with higher degrees of platelet reactivity compared with patients with normal renal function/mild CKD. In partic-

Table 2 **Platelet Activation Profiles According to HPPR Status**

	$HPPR_{ADP}$			$HPPR_{COLL}$			$HPPR_{ADP+COLL}$		
	Yes	No	p Value	Yes	No	p Value	Yes	No	p Value
Antifibrinogen	37 ± 20	27 ± 19	<0.001	37 ± 20	28 ± 20	0.001	40 ± 19	28 ± 20	<0.001
PAC-1	49 ± 19	34 ± 20	<0.001	44 ± 19	36 ± 21	0.004	48 ± 19	36 ± 20	<0.001
P-selectin	44 ± 18	31 ± 18	<0.001	37 ± 19	33 ± 19	0.19	43 ± 19	33 ± 19	0.001

Values are mean ± SD. High post-treatment platelet reactivity to ADP- ($HPPR_{ADP}$), collagen- ($HPPR_{COLL}$), and both ADP- and collagen-induced ($HPPR_{ADP+COLL}$) aggregation. PAC-1 (PAC-1 FICT conjugated; Becton Dickinson, Rutherford, New Jersey).

ADP = adenosine disphosphate; COLL = collagen HPPR = high post-treatment platelet reactivity.

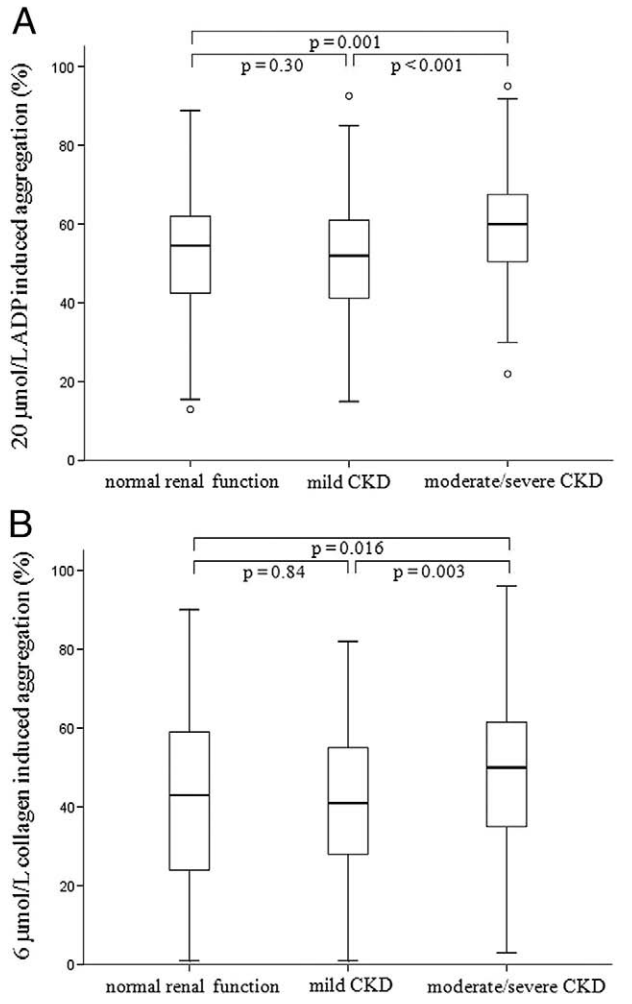

Figure 2 **Platelet Aggregation According to Renal Function**

Box-and-whisker plot demonstrating post-treatment adenosine diphosphate (ADP)-induced **(A)** and collagen-induced **(B)** platelet aggregation as measured by light transmittance aggregometry in patients with creatinine clearance ≥90 ml/min (normal), 60 to 89 ml/min (mild chronic kidney disease [CKD]), and <60 ml/min (moderate/severe CKD) on chronic clopidogrel therapy. The **central box** represents the values between the lower and upper quartiles, and the **middle line** is the median. The **vertical line** extends to 1.5 interquartile ranges from both ends of the box, excluding outliers (values more than 1.5 interquartile ranges from the end of the box), which are displayed as separate points.

ular, after adjustment for potential confounders, patients with creatinine clearance <60 ml/min had an almost 4-fold increase in the likelihood of showing high platelet reactivity after ADP stimuli and over a 2-fold increase in high platelet reactivity after collagen stimuli. Importantly, these patients with high platelet aggregability also have increased markers of platelet activation. Overall, these findings are indicative not only of dysfunctional purinergic signaling mediated ADP receptors but also of the presence of a hyper-reactive platelet phenotype with upregulation of multiple signaling pathways. Therefore, these pharmacodynamic observations might explain the elevated prevalence of ischemic complications, including stent thrombosis, among DM patients with advanced CKD.

Diabetes mellitus is the single most important cause of CKD (17–19). One year after successful PCI, mortality is 5-fold higher in patients with moderate CKD and 12-fold higher in patients with severe CKD than in those with normal renal function (6). The CKD patients also have more aggressive atherosclerotic disease and a greater risk of thrombotic complications, including stent thrombosis (5,7,8,10–14). Therefore, it might be expected that CKD patients are more likely to derive enhanced benefit from therapeutic approaches shown to improve cardiovascular outcomes in high-risk patients, including more potent antiplatelet treatment regimens as with adjunctive clopidogrel therapy. However, a post hoc analysis of the CREDO (Clopidogrel for the Reduction of Events During Observation) trial showed that, although long-term clopidogrel use in patients with normal renal function resulted in a significant reduction in major adverse cardiovascular events at 28 days and 1 year, this benefit was less apparent in mild CKD and not apparent at all in moderate CKD patients (15). More recently, a post hoc analysis of the CHARISMA (Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management, and Avoidance) trial showed that clopidogrel compared with placebo might actually be harmful (increased cardiovascular and overall mortality) in patients with diabetic nephropathy, claiming the need for additional studies to investigate this possible interaction (16).

To the best of our knowledge, this is the first study providing a potential underlying mechanism to explain why DM patients with CKD have worse outcomes. The current pharmacodynamic analysis demonstrates a positive relation between moderate/severe CKD and HPPR in aspirin-treated DM patients receiving adjunctive clopidogrel therapy. This was confirmed even after adjustment for potential confounding factors. In particular, we showed that differences in platelet reactivity while receiving standard dual antiplatelet therapy occurred only in patients with moderate/severe CKD, whereas no differences were noted in patients with mild CKD or normal renal function. Whether severe CKD is associated with worse outcomes than moderate CKD cannot be addressed by these data, because patients with moderate or severe CKD were combined into 1 single group for the purposes of the planned comparisons. Interestingly, Deray et al. (33) have previously reported in a small cohort the lack of difference in platelet response to clopidogrel between patients with moderate and those with severe CKD. These observations in aggregate suggest the presence of a threshold of renal function below which a greater degree of platelet reactivity occurs. This is in agreement with clinical studies in patients with dual antiplatelet therapy in which adverse outcomes dramatically increased in patients with more advanced stages of CKD (10,15,16). This also concurs with pharmacodynamic assessments, including those conducted in DM patients re-

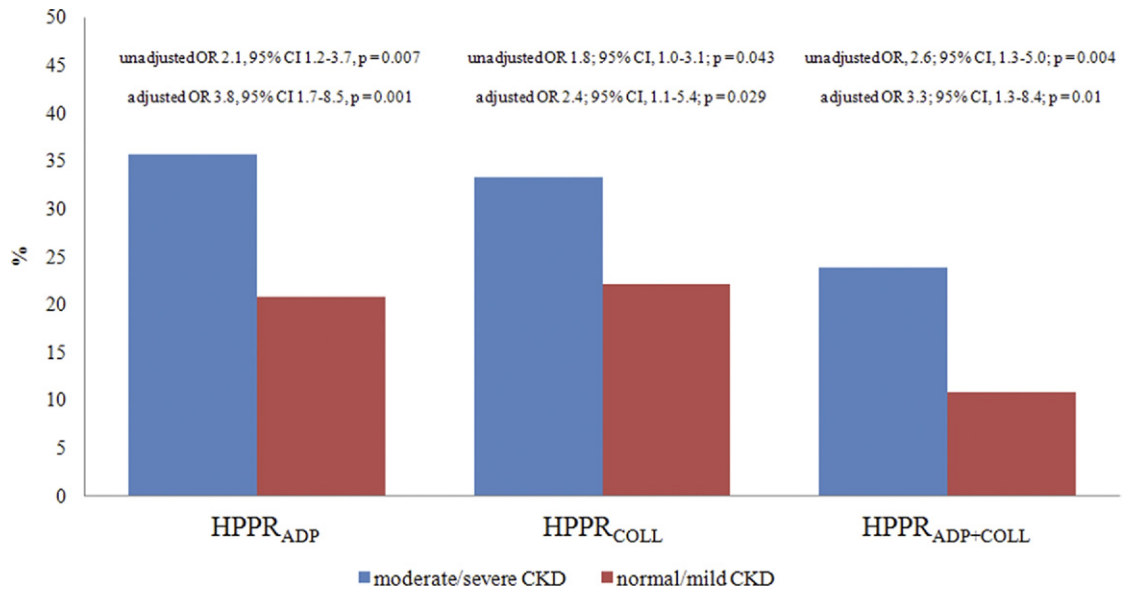

Figure 3 **Prevalence of Moderate/Severe CKD and Normal Renal Function/Mild CKD in Patients With HPPR**

Prevalence of moderate/severe chronic kidney disease (CKD) and normal renal function/mild CKD in patients with high post-treatment platelet reactivity (HPPR) according to adenosine diphosphate (ADP)- (HPPR$_{ADP}$), collagen- (HPPR$_{COLL}$), or both ADP- and collagen-induced (HPPR$_{ADP+COLL}$) aggregation. CI = confidence interval; OR = odds ratio.

ceiving dual antiplatelet therapy, showing that long-term adverse outcomes markedly increased above a certain threshold of platelet reactivity (29,32,34–36).

Although multiple mechanisms have been implied in heightened platelet reactivity in DM patients, it is unknown why the presence of CKD in these patients further adds to their degree of platelet dysfunction. Indeed, CKD per se has been shown to be associated with platelet abnormalities (37–41). However, contrary to pharmacodynamic studies performed in DM patients consistently demonstrating heightened platelet reactivity, reports in CKD patients have shown contrasting data. In fact, in some of these studies platelet reactivity was shown to be depressed, whereas in others platelet reactivity was shown to be increased (37–41). These conflicting findings might be attributed to patient selection, given that the presence of DM—known to have specific abnormalities in their platelet biology—was not specified.

There has been accumulating data supporting the prognostic implications of HPPR in patients receiving dual antiplatelet therapy (27). In particular, HPPR is associated with a higher risk of recurrent ischemic events, including stent thrombosis. Although patients with DM receiving dual antiplatelet therapy are known to have higher platelet reactivity compared with non-DM (21,23,25,29), this study shows a broad variability in their platelet function profiles, and the presence of moderate/severe CKD allows for identification of those DM patients with greater likelihood of having HPPR. These pharmacodynamic findings might contribute to explain why some DM patients have worse outcomes than others and suggest the potential need for further categorizing this already high-risk cohort of patients into those with and without CKD. This might enable a better understanding of their individual risk profile and allow the future development of targeted treatment strategies for these patients.

The persistence of high platelet reactivity despite adjunctive clopidogrel therapy underscores the need for more potent platelet inhibiting strategies to reduce recurrent ischemic event risk, including high clopidogrel dosing and novel antiplatelet agents (23,42). However, it may be argued that high platelet reactivity alone might not be a good indicator for using more potent platelet inhibition, because their potential benefit of reducing ischemic events might be offset by increased bleeding rates if CKD is also present. In fact, impaired renal function is well-established to be associated with increased bleeding risk (12,43–46). This is of clinical relevance, given that approximately 50% of CKD patients have DM. However, the TRITON–TIMI 38 trial (TRial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet InhibitioN with Prasugrel–Thrombolysis In Myocardial Infarction 38) showed that renal failure was not a predictor for increased bleeding in patients treated with prasugrel, a third-generation thienopyridine with potent P2Y$_{12}$ inhibitory effects (47). Importantly, prasugrel achieved its greatest clinical benefit in DM patients without increasing the risk of major bleeding compared with clopidogrel (48). Indeed, evaluating outcome data in DM patients according to the presence or absence of CKD will provide more insights on the risk-benefit

ratio of antithrombotic strategies, particularly with the introduction of novel and more potent agents.

Study limitations. This is a cross-sectional study of independent groups and suffers from the obvious limitations of a nonrandomized trial. In an attempt to account for these limitations, we also made comparisons that were adjusted for several clinical variables. Due to the large number of variables introduced, we cannot exclude a potential for overfitting of the model. Furthermore, the independent effect of renal function in DM patients with CAD has been investigated while participants were already receiving dual antiplatelet therapy. Although absolute platelet reactivity values have shown to be of greater prognostic significance compared with relative changes (27), the latter could have provided more insights on $P2Y_{12}$ signaling in CKD patients. Also, factors such as fibrinogen levels have shown to influence platelet reactivity in DM patients (49), but whether these have an impact in the DM population with CKD was not explored in this study. Therefore, dedicated studies are warranted to better understand $P2Y_{12}$-mediated signaling in platelets from CKD patients. Ultimately, whether the findings of our study can be extrapolated to CKD patients without DM remains unknown.

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