Original Article

Clopidogrel Withdrawal Is Associated With Proinflammatory and Prothrombotic Effects in Patients With Diabetes and Coronary Artery Disease

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Inhibition of the $P2Y_{12}$ pathway by the platelet antagonist clopidogrel is associated with a marked reduction in platelet reactivity. Recent reports have shown that $P2Y_{12}$ inhibition has anti-inflammatory effects as well. However, whether clopidogrel withdrawal is associated with proaggregatory and proinflammatory effects has not yet been explored. Since diabetic subjects are characterized by a prothrombotic and proinflammatory status, we hypothesize that these patients may be more vulnerable to these effects. A total 54 patients with diabetes on long-term (12 months) dual antiplatelet therapy (aspirin plus clopidogrel) were studied. Platelet aggregation (following 6 and 20 μmol/l ADP stimuli) and inflammatory markers (C-reactive protein and P-selectin expression) were assessed before and 1 month following clopidogrel withdrawal. Following clopidogrel withdrawal, aspirin responsiveness using platelet function analyzer–100 was determined as well. A significant increase in all the assessed platelet ($P < 0.0001$ for 6 and 20 μmol/l ADP-induced aggregation) and inflammatory ($P < 0.05$ for C-reactive protein, $P < 0.001$ for P-selectin expression in resting platelets, and $P < 0.0001$ for P-selectin expression in ADP-stimulated platelets) biomarkers was observed following clopidogrel withdrawal. Low responders to aspirin had increased platelet aggregation profiles ($P < 0.05$ for 6 and 20 μmol/l ADP-induced aggregation) but no differences in inflammatory markers. In conclusion, clopidogrel withdrawal is associated with an increase in platelet and inflammatory biomarkers in diabetic patients, supporting pleiotropic effects coupled with $P2Y_{12}$ receptor antagonism. *Diabetes* 55: 780–784, 2006

The addition of clopidogrel to aspirin is associated with a marked reduction in platelet reactivity, which represents the main mechanism through which dual antiplatelet therapy exerts its clinical benefits (1–4). Such clinical benefits may also be attributed in part to the fact that many patients have abnormally high platelet reactivity despite being treated with aspirin; these patients are termed "low responders" and have a higher risk of ischemic events (5). Further, recent reports have shown that clopidogrel has anti-inflammatory properties as well; therefore, clopidogrel may exert its beneficial effects by "cooling-off" two important pathogenetic pathways, platelet reactivity and inflammation, both deeply involved in atherothrombotic disease (6–10).

In patients undergoing percutaneous coronary intervention (PCI), current recommendations limit the use of a dual antiplatelet regimen to 1 year, following which patients withdraw clopidogrel and indefinitely maintain treatment with aspirin (11). Although numerous reports have focused on the functional implications on inflammation and platelet reactivity achieved with the addition of clopidogrel, how these may be affected by its withdrawal is unknown. Notably, patients with diabetes have increased platelet reactivity and a proinflammatory status, which explains why they highly benefit from antiplatelet therapy and also why they are more susceptible to atherothrombotic events in the absence of antiplatelet therapy (12,13). In the present report, we assessed how clopidogrel withdrawal influences markers of inflammation and platelet aggregation in diabetic patients and how individual responsiveness to aspirin may impact these biomarkers post–clopidogrel withdrawal.

RESEARCH DESIGN AND METHODS

A total of 54 patients with diabetes were studied. Type 1 and type 2 diabetes were defined according to the World Health Organization report (14), and diet-controlled diabetic patients were excluded. Arterial hypertension was defined as a systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg on two or more separate occasions or as the use of antihypertensive agents. Hypercholesterolemia was defined as a total cholesterol level ≥240 mg/dl or as the use of a lipid-lowering agent. All patients had previously undergone PCI and were treated with clopidogrel (75 mg/day) for 1 year. All patients were concomitantly treated with the same dose of aspirin (100 mg/day). After 1 year, clopidogrel was withdrawn and patients maintained aspirin indefinitely. Blood sampling was performed at their 1-year PCI clinical follow-up (study time-point 1: patients on aspirin and clopidogrel) and

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Received for publication 26 October 2005 and accepted in revised form 30 November 2005.

CEPI, collagen and epinephrine; CT, closure time; hs-CRP, high-sensitivity C-reactive protein; PCI, percutaneous coronary intervention; PFA, platelet function analyzer; PPP, platelet-poor plasma; PRP, platelet-rich plasma.

1 month after clopidogrel withdrawal (study time-point 2: patients only on aspirin). Blood sampling was performed 2–4 h after antiplatelet drug intake, and markers of inflammation and platelet aggregation were assessed at both time points. Following clopidogrel withdrawal, responsiveness to aspirin was assessed as well. Patients who were on other antithrombotic drugs (oral anticoagulants, dypiridamole, or cilostazol) or who had presented with an acute ischemic event over the previous 12 months were not eligible for the study in order to avoid confounders on the biomarker assessments.

Inflammatory and platelet biomarker assessments. Inflammatory markers included high-sensitivity C-reactive protein (hs-CRP) and P-selectin expression. Hs-CRP was quantified by kinetic nephelometry with an immunochemical system (IMMAGE CRPH; Beckman Coulter). Cell-surface P-selectin expression (CD62-P) was measured by whole-blood cytometry before and after 2 μmol/l ADP stimuli (ChronoLog, Havertown, PA), as previously described (3,4). In brief, P-selectin expression was assessed using a phycoerithrin-conjugated anti-CD62P antibody (final concentration 0.3 mg/l; Becton Dickinson, San José, CA). An EPICS-XL PROFILE II flow cytometer (Coulter, Miami, FL) was used for the assessment. Samples were analyzed within 2 h by flow cytometry, and platelets were identified based on particle size (forward scatter) and complexity (side scatter). Light scatter and fluorescence data from 10,000 platelet events were collected with all detectors in logarithmic mode. Acquisition and processing data were analyzed with XL2 software (Coulter). P-selectin expression was expressed as the percentage of platelets positive for antibody binding.

Platelet aggregation was assessed using platelet-rich plasma (PRP) by the turbidimetric method in a two-channel aggregometer (490 model; Chrono-Log, Havertown, PA), as previously described (3,4). Platelet agonists included 6 and 20 μmol/l ADP (Chrono-Log). PRP was obtained as a supernatant after centrifugation of citrated blood at 800 rpm for 10 min. Platelet-poor plasma (PPP) was obtained by a second centrifugation of the blood fraction at 2,500 rpm for 10 min. The platelet count in PRP was adjusted to the range of 250.000/μl by dilution with autologous plasma when the platelet count was out of range. Light transmission was adjusted to 0% with PRP and to 100% for PPP for each measurement. Platelet aggregation was assessed within 2 h from blood sampling. PRP was kept at 22°C before use and at 37°C 1 min before running the aggregatory test. Aggregation was assessed in siliconized tubes at 37°C in constant stirring conditions, and curves were recorded for 5 min. Platelet aggregation was determined as the maximal percentage of change in light transmittance from baseline using PPP as the reference.

Responsiveness to aspirin was assessed using the platelet function analyzer (PFA)-100 system (Dade-Behring International, Miami, FL). The PFA-100 is a microprocessor-controlled instrument/test cartridge system used to assess platelet function simulating platelet-based primary hemostasis in vitro (15). A syringe aspirates citrated whole blood under high shear-flow conditions (5,000–6,000 s^{-1}) through a small aperture (150 μm) cut into a membrane placed in the test cartridge. Sensitivity to aspirin is assessed by cartridges presenting a membrane coated with type I collagen and 10 μg epinephrine bitartrate (collagen and epinephrine [CEPI]). The time necessary for the occlusion of the aperture, defined as closure time (CT), is indicative of platelet reactivity on the whole blood sample. CEPI-CT ranges between 94 and 193 s and is prolonged in patients treated with aspirin (15,16). After 300 s the process automatically terminates. A CEPI-CT ≤193 s (upper normal range limit) has been considered as a cut-off value to define low responders to aspirin and was used accordingly in the present study (15,16).

Statistical analysis. Variables were analyzed for a normal distribution with the Kolmogorov-Smirnov test. Normally distributed variables are presented as means ± SD. Variables that did not follow a normal distribution are represented as median and interquartile range. Categorical variables are expressed as frequencies and percentages. The paired-samples Student's *t* test was used for normally distributed continuous variables and the sign and median tests if not normally distributed. Categorical variables were compared by means of the χ^2 test or Fisher's exact test when at least 25% of values showed an expected cell frequency of less than five. For the analysis of correlation between two quantitative variables, the Pearson test was used. A P value <0.05 was considered statistically significant. Statistical analysis was performed using a SPSS version 11.0 software (SPSS, Chicago, IL).

RESULTS

Baseline characteristics and demographics of the study population are described in Table 1. Following clopidogrel withdrawal, there was a significant increase in markers of inflammation ($P < 0.05$ for hs-CRP, $P < 0.001$ for P-selectin expression in resting platelets, and $P < 0.0001$ for P-selectin expression in ADP-stimulated platelets; Fig. 1). Platelet aggregation profiles also increased significantly following clopidogrel withdrawal ($P < 0.0001$ and $P < 0.0001$ for 6 and 20 μmol/l ADP, respectively; Fig. 2). There were no differences in hematocrit (40.5 ± 3.8 vs. 41.2 ± 3.3%), platelet count (222.3 ± 58.9 vs. 232.2 ± 51.5 10^9/ml), and mean platelet volume (8.9 ± 1.0 vs. 8.8 ± 1.2 fl) before and after clopidogrel withdrawal. A1C levels (7.1 ± 1.3 and 7.1 ± 1.9% before and after clopidogrel withdrawal, respectively) did not correlate with inflammatory or platelet biomarkers.

TABLE 1
Baseline demographics of the study population

n	54
Age (years)	67 ± 8
Male sex	29 (54)
Risk factors	
Smoking	5 (9)
Hyperlipemia	40 (74)
Hypertension	37 (69)
Type 1 diabetes	14 (26)
Type 2 diabetes	40 (74)
Treatment	
β-Blockers	38 (70)
Nitrates	23 (35)
ACE inhibitors	29 (54)
Statins	44 (82)
Calcium blockers	16 (30)

Data are means ± SD and *n* (%).

In the overall study population, 24 patients (44%) were low responders to aspirin (CEPI-CT ≤193 s). These patients had baseline characteristics and demographics similar to those of patients with more optimal responsiveness to aspirin ($n = 30$). Following clopidogrel withdrawal, low responders to aspirin had increased platelet aggregation profiles compared with aspirin-sensitive patients ($P = 0.036$ and $P = 0.01$ for 6 and 20 μmol/l ADP, respectively; Fig. 3). No differences were observed in platelet aggregation profiles in these patients when they were concomitantly treated with clopidogrel ($P = 0.4$ and $P = 0.3$ for 6 and 20 μmol/l ADP, respectively; Fig. 3). Inflammatory markers were not affected by sensitivity to aspirin: median 0.45 (interquartile range 0.23–0.73) vs. 0.34 (0.17–0.79), $P = 0.46$ for hs-CRP; 29.9 ± 22.0 vs. 28.2 ± 25.0, $P = 0.78$ for P-selectin expression in resting platelets; and 62.8 ± 17.5 vs. 55.5 ± 19.7, $P = 0.16$ for P-selectin expression in ADP-stimulated platelets.

DISCUSSION

This is the first report to assess the effects of clopidogrel withdrawal on inflammatory and platelet biomarkers in patients on long-term dual antiplatelet therapy. The main finding of this study is that diabetic patients on long-term dual antiplatelet therapy experience proinflammatory and prothrombotic effects following clopidogrel withdrawal. In addition, low responders to aspirin identified using a point-of-care assessment showed increased platelet aggregation profiles.

Several reports have shown that withdrawal of oral antiplatelet agents, in particular aspirin, is associated with an increased risk of death and myocardial infarction, likely related to a rebound in platelet reactivity (17–19). In the current era of drug-eluting stents, there is a growing concern with regards to withdrawal of oral antiplatelet therapy, in particular clopidogrel, since this has been highly associated with stent thrombosis (20). In a series of

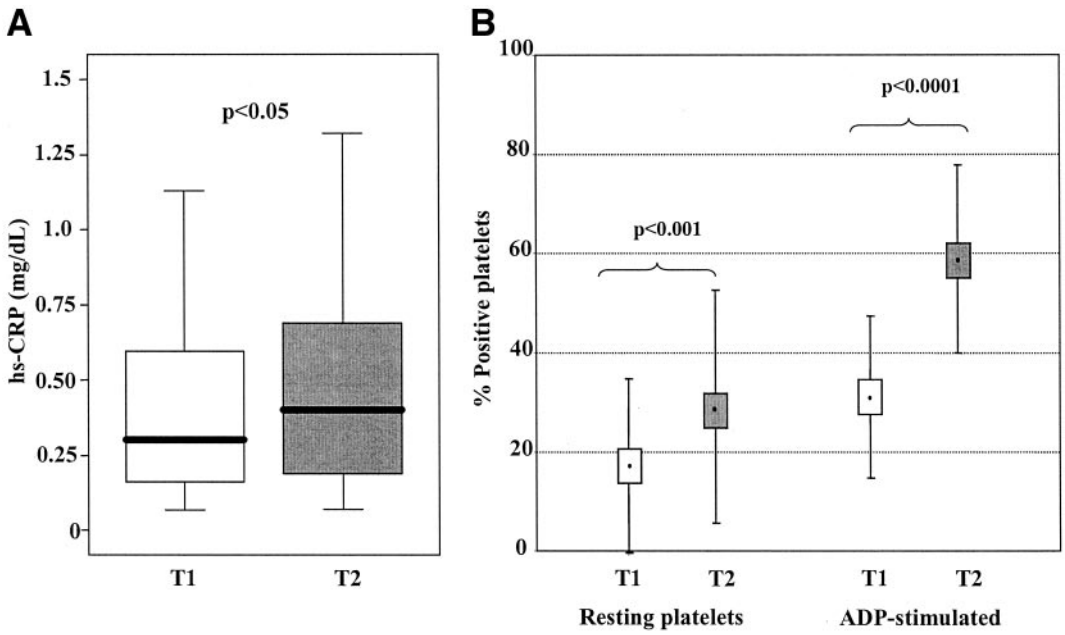

FIG. 1. hs-CRP (*A*) and P-selectin (*B*) in resting platelets and P-selectin in ADP-stimulated platelets before (T1: patients on aspirin and clopidogrel) and 1 month after (T2: patients only on aspirin) clopidogrel withdrawal. Hs-CRP levels are expressed in mg/dl (median [interquartile range]). P-selectin is expressed as the percentage of positive platelets (means ± SD).

2,229 patients, Iakavou et al. (20) showed that premature discontinuation of oral antiplatelet agents was the most important predictor of stent thrombosis. Diabetes is another independent predictor of stent thrombosis (20). The increase in proaggregatory and proinflammatory profiles (both intrinsically associated with thrombotic formation) found in our functional study may therefore be the explanatory mechanisms that contribute to this undesired event.

Another finding from this study is the observation that patients with low responsiveness to aspirin are more likely to have higher platelet aggregation profiles. This may explain why stent thrombosis occurs only in selected patients after clopidogrel withdrawal. The observation that patients with diabetes frequently have suboptimal response to aspirin may also be implicated in their increased thrombotic event rate, which has been invoked as a potential mechanism leading to stent thrombosis occurring even after 1 year of clopidogrel treatment (21–25). Several mechanisms lead to reduced responsiveness to aspirin and increased platelet reactivity in diabetic patients, including increased platelet exposure to ADP (21). This may explain why the use of the ADP receptor antagonist clopidogrel is superior to aspirin in secondary prevention of ischemic events in diabetic patients (26). Although patients with reduced sensitivity to aspirin identified while concomitantly treated with clopidogrel have been shown to have increased platelet reactivity compared with aspirin-responsive patients (27), notably in this study we observed that patients with reduced aspirin sensitivity who were identified following clopidogrel withdrawal had no significant differences in platelet aggregation when treated with clopidogrel. Overall, these findings suggest

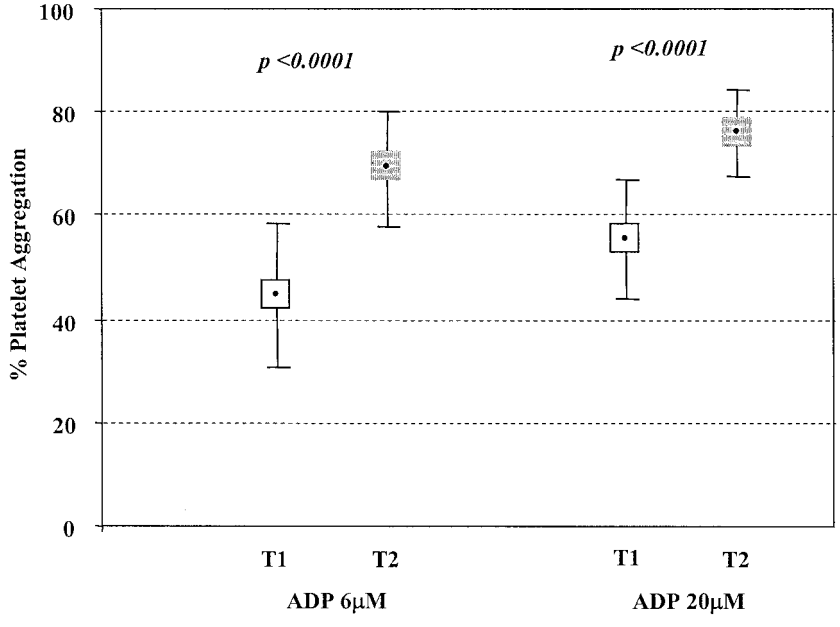

FIG. 2. Platelet aggregation in 6 and 20 μmol/l ADP-stimulated platelets before (T1: patients on aspirin and clopidogrel) and 1 month after (T2: patients only on aspirin) clopidogrel withdrawal. Data are expressed as percentage of maximal platelet aggregation (means ± SD).

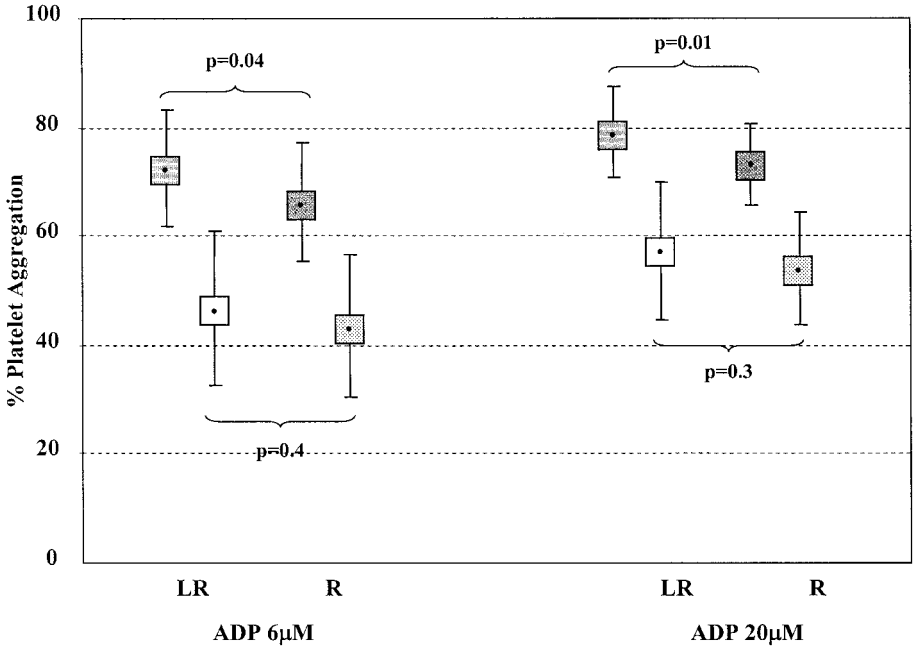

FIG. 3. Platelet aggregation in 6 and 20 μmol/l ADP-stimulated platelets in responders (R) and low responders (LR) to aspirin before (open boxes, patients on aspirin and clopidogrel) and 1 month after (shaded boxes, patients only on aspirin) clopidogrel withdrawal. Data are expressed as percentage of maximal platelet aggregation (means ± SD).

that clopidogrel may hamper the effects of increased ADP exposure, although not overcome other mechanisms involved in the aspirin resistance phenomenon. Whether clopidogrel will improve clinical outcomes in low responders to aspirin is still unknown and is currently being evaluated in the ongoing CHARISMA (Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization Management and Avoidance) trial, which includes over 6,000 patients with diabetes (28).

Currently, several techniques are available to assess aspirin responsiveness, and the prevalence of low responders to aspirin may vary according to the sensitivity of the test used (5). The high sensitivity of the PFA-100 system explains why the prevalence of low responders is elevated when using this technique (29). In fact, PFA-100 assesses platelet reactivity in whole blood; therefore, individual responsiveness to aspirin is strongly affected by pathways of platelet activation other than cyclooxygenease-1 (29). The pathways extrinsic to the cyclooxygenease-1 enzyme are highly implicated in the diabetic platelet, overestimating the incidence of the aspirin resistance phenomenon (21,29). However, our results compare favorably with those previously described using the same methodology (30). Despite the limitations of systems that do not measure cyclooxygenease-1 inhibition directly, but rather measure platelet reactivity such as PFA-100, these techniques present the advantage of being simple and rapid to use as well as widely available compared with other techniques, such as traditional aggregometry or thromboxane metabolite assessment. Therefore, the prognostic implications associated with increased platelet reactivity make PFA-100 a useful tool for risk stratification to define patients with a prothrombotic status and who may potentially benefit from more aggressive antithrombotic regimens (31).

The lack of differences in inflammatory markers based on individual responsiveness to aspirin, in particular hs-CRP and P-selectin, is in line with the previous demonstration that aspirin does not affect either of these markers (32–34). On the contrary, adjunctive treatment with clopidogrel has been associated with a reduction in these markers, which have both been associated with worse outcomes, even after PCI (35–36). These anti-inflammatory effects may therefore be specifically linked to the ADP P_2Y_{12} receptor antagonized by clopidogrel (6–9). Cumulatively, these findings support the hypothesis that clopidogrel is not just an antiplatelet agent, but a drug with pleiotropic effects, which contributes to its long-term benefits. The impact of clopidogrel on inflammatory markers and how this may affect clinical outcomes is also being evaluated in the CHARISMA trial (28).

In conclusion, clopidogrel withdrawal is associated with proinflammatory and prothrombotic effects in patients with diabetes, supporting the pleiotropic properties coupled with P_2Y_{12} receptor antagonism. The prognostic implications associated with increased platelet reactivity and enhanced inflammatory status, which include progression of atherosclerotic disease and atherothrombotic complications, underscores the potential clinical impact of these functional findings (23–25,35–39). The influence of clopidogrel on inflammatory markers and platelet biomarkers and how these may affect long-term clinical outcomes is currently being evaluated in a large-scale randomized clinical trial.

Study limitations. The lack of samples before clopidogrel administration is a limitation to this study, and it would have been of adjunctive value to define the extent to which our findings represent a "rebound" phenomenon as well as to better define responsiveness to antiplatelet treatment. Nevertheless, the overall degree of post-treatment platelet reactivity rather than responsiveness appears to be more important to predict future ischemic risk (39–40). In addition, this study was carried out only in diabetic patients, and whether proinflammatory and proaggregatory effects following clopidogrel withdrawal may be found in nondiabetic patients cannot be extrapolated from this functional study. Nevertheless, homogeneous, albeit small, patient populations are mandatory to better assess these functional implications. Ultimately, larger scale studies are warranted to assess the clinical implications of the surrogate markers assessed in the specific scenario of our study.

REFERENCES

1. Yusuf S, Zhao F, Mehta SR, Chrolavicius S, Tognoni G, Fox KK: Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST segment elevation. *N Engl J Med* 345:494–502, 2001
2. Steinhubl SR, Berger PB, Mann JT 3rd, Fry ET, DeLago A, Wilmer C, Topol EJ: Early and sustained dual oral antiplatelet therapy following percutaneous coronary intervention: a randomized controlled trial. *JAMA* 288: 2411–2420, 2002
3. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Ramirez C, Barrera-Ramirez C, Sabate M, Hernandez R, Moreno R, Escaned J, Alfonso F, Banuelos C, Costa MA, Bass TA, Macaya C: Identification of low responders to a 300 mg clopidogrel loading dose in patients undergoing coronary stenting. *Thromb Res* 115:101–118, 2005
4. Angiolillo DJ, Fernández-Ortiz A, Bernardo E, Ramirez C, Sabate M, Banuelos C, Hernandez-Antolin R, Escaned J, Moreno R, Alfonso F, Macaya C: Clopidogrel responders and interindividual variability in platelet inhibition following a high clopidogrel loading dose regimen during coronary intervention. *Eur Heart J* 25:1903–1910, 2004
5. Mason PJ, Jacobs AK, Freedman JE: Aspirin resistance and atherothrombotic disease. *J Am Coll Cardiol* 46:986–993, 2005
6. Vivekananthan DP, Bhatt DL, Chew DP, Zidar FJ, Chan AW, Moliterno DJ, Ellis SG, Topol EJ: Effect of clopidogrel pretreatment on periprocedural rise in C-reactive protein after percutaneous coronary intervention. *Am J Cardiol* 94:358–360, 2004
7. Molero L, Lopez-Farre A, Mateos-Caceres PJ, Fernandez-Sanchez R, Luisa Maestro M, Silva J, Rodriguez E, Macaya C: Effect of clopidogrel on the expression of inflammatory markers in rabbit ischemic coronary artery. *Br J Pharmacol* 146:419–424, 2005
8. Xiao Z, Theroux P: Clopidogrel inhibits platelet-leukocyte interactions and thrombin receptor agonist peptide-induced platelet activation in patients with an acute coronary syndrome. *J Am Coll Cardiol* 43:1982–1988, 2004
9. Quinn MJ, Bhatt DL, Zidar F, Vivekananthan D, Chew DP, Ellis SG, Plow E, Topol EJ: Effect of clopidogrel pretreatment on inflammatory marker expression in patients undergoing percutaneous coronary intervention. *Am J Cardiol* 93:679–684, 2004
10. Cha JK, Jeong MH, Lee KM, Bae HR, Lim YJ, Park KW, Cheon SW: Changes in platelet P-selectin and in plasma C-reactive protein in acute atherosclerotic ischemic stroke treated with a loading dose of clopidogrel. *J Thromb Thrombolysis* 14:145–150, 2002
11. Popma JJ, Berger P, Ohman EM, Harrington RA, Grines C, Weitz JI Antithrombotic therapy during percutaneous coronary intervention: the Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. *Chest* 126 (Suppl. 3):576S–599S, 2004
12. Biondi-Zoccai GG, Abate A, Liuzzo G, Biasucci LM: Atherothrombosis, inflammation, and diabetes. *J Am Coll Cardiol* 41:1071–1077, 2003
13. Colwell RW, Nesto JA: The platelet in diabetes: focus on prevention of ischemic events. *Diabetes Care* 26:2181–2188, 2003
14. Alberti KGMM, Zimmet PZ: Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1. Diagnosis and classification of diabetes mellitus: provisional report of a WHO consultation. *Diabet Med* 15:539–553, 1998
15. Favaloro EJ: Clinical application of the PFA-100. *Curr Opin Hematol* 9:407–415, 2002
16. Gum PA, Kottke-Marchant K, Poggio ED, Gurm H, Welsh PA, Brooks L, Sapp SK, Topol EJ: Profile and prevalence of aspirin resistance in patients with cardiovascular disease. *Am J Cardiol* 88:230–235, 2001
17. Collet JP, Montalescot G, Blanchet B, Tanguy ML, Golmard JL, Choussat R, Beygui, Payot L, Vignolles N, Metzger JP, Thomas D: Impact of prior use or recent withdrawal of oral antiplatelet agents on acute coronary syndromes. *Circulation* 110:2361–2367, 2004
18. Collet JP, Himbert D, Steg PG: Myocardial infarction after aspirin cessation in stable coronary artery disease patients (Letter). *Int J Cardiol* 76:257–258, 2000
19. Vial JH, McLeod LJ, Roberts MS: Rebound elevation in urinary thromboxane B2 and 6-keto-PFF1 alpha exacerbation after aspirin withdrawal. *Adv Prostaglandin Thromboxane Leukot Res* 21A:157–160, 1991
20. Iakovou I, Schidt T, Bonizzoni E, Ge L, Sangiorgi GM, Stankovic G, Airoldi F, Chieffo A, Montorfano M, Carlino M, Michev I, Corvaja N, Briguori C, Gerckens U, Grube E, Colombo A: Incidence, predictors, and outcome of thrombosis after successful implantation of drug-eluting stents. *JAMA* 293:2126–2130, 2005
21. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Ramirez C, Sabate M, Jimenez-Quevedo P, Hernandez R, Moreno R, Escaned J, Alfonso F, Banuelos C, Costa MA, Bass TA, Macaya C: Platelet function profiles in patients with type 2 diabetes and coronary artery disease on combined aspirin and clopidogrel treatment. *Diabetes* 54:2430–2435, 2005
22. Jimenez-Quevedo P, Angiolillo DJ, Bernardo E, Sabate M: Late stent thrombosis (> 1 year) following clopidogrel withdrawal after brachytherapy treatment: need to assess aspirin resistance? *Catheter Cardiovasc Interv* 62:39–42, 2004
23. Ajzenberg N, Aubry P, Huisse MG, Cachier A, El Amara W, Feldman LJ, Himbert D, Baruch D, Guillin MC, Steg PG: Enhanced shear-induced platelet aggregation in patients who experience subacute stent thrombosis: a case-control study. *J Am Coll Cardiol* 45:1753–1756, 2005
24. Wenawesser P, Dorffler-Melly, Imboden K, Windecker S, Togni M, Meier B, Haeberli A, Hess OM: Stent thrombosis is associated with an impaired response to antiplatelet therapy. *J Am Coll Cardiol* 45:1748–1752, 2005
25. Gurbel PA, Bliden KP, Samara W, Yoho JA, Hayes K, Fissha MZ, Tantry US: Clopidogrel effect on platelet reactivity in patients with stent thrombosis: results of the CREST study. *J Am Coll Cardiol* 46:1827–1832, 2005
26. Bhatt DL, Marso SP, Hirsch AT, Ringleb PA, Hacke W, Topol EJ: Amplified benefit of clopidogrel versus aspirin in patients with diabetes mellitus. *Am J Cardiol* 90:625–628, 2002
27. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Ramirez C, Sabate M, Jimenez-Quevedo P, Hernandez R, Moreno R, Escaned J, Alfonso F, Banuelos C, Costa MA, Bass TA, Macaya C: Influence of aspirin resistance on platelet function profiles in patients on long-term aspirin and clopidogrel after percutaneous coronary intervention. *Am J Cardiol* 97:38–43, 2006
28. Bhatt DL, Topol EJ, the CHARISMA Executive Committee: Clopidogrel added to aspirin versus aspirin alone in secondary prevention and high-risk primary prevention: rationale and design of the Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management, and Avoidance (CHARISMA) trial. *Am Heart J* 148:263–268, 2004
29. Tantry US, Bliden KP, Gurbel PA: Overestimation of platelet aspirin resistance detection by thrombelastograph platelet mapping and validation by conventional aggregometry using arachidonic acid stimulation. *J Am Coll Cardiol* 46:1705–1709, 2005
30. Fateh-Moghadam S, Plockinger U, Cabeza N, Htun P, Reuter T, Ersel S, Gawaz M, Dietz R, Bocksch W: Prevalence of aspirin resistance in patients with type 2 diabetes. *Acta Diabetol* 42:99–103, 2005
31. Frossard M, Fuchs, Leitner JM, Hsieh K, Vlcek M, Losert H, Domanovitis H, Schreiber W, Laggner AN, Jilma B: Platelet function predicts myocardial damage in patients with acute myocardial infarction. *Circulation* 110: 1392–1397, 2004
32. Feldman M, Jialal I, Devaraj S, Cryer B: Effects of low-dose aspirin on serum C-reactive protein and thromboxane B2 concentrations: a placebo-controlled study using a highly sensitive C-reactive protein assay. *J Am Coll Cardiol* 37:2036–2041, 2001
33. Feng DL, Tracy RP, Lipinska I, Murillo J, McKenna C, Tofler GH: Effect of short-term aspirin use on C-reactive protein. *J Thromb Thrombol* 9:37–41, 2000
34. Klinkhardt U, Graff J, Harder S: Clopidogrel, but not abciximab, reduces platelet leukocyte conjugates and P-selectin expression in a human ex vivo in vitro model. *Clin Pharmacol Ther* 71:176–185, 2002
35. Tschoepe D, Schultheiss HP, Kolarov P, Schwippert B, Dannehl K, Nieuwenhuis HK, Kehrel B, Strauer B, Gries FA: Platelet membrane activation markers are predictive for increased risk of acute ischemic events after PTCA. *Circulation* 88:37–42, 1993
36. Buffon A, Liuzzo G, Biasucci LM, Pasqualetti P, Ramazzotti V, Rebuzzi AG, Crea F, Maseri A: Preprocedural serum levels of C-reactive protein predict early complications and late restenosis after coronary angioplasty. *J Am Coll Cardiol* 34:1512–1521, 1999
37. Kabbani SS, Watkins MW, Ashikaga T, Terrien EF, Holoch PA, Sobel BE, Schneider DJ: Platelet reactivity characterized prospectively: a determinant of outcome 90 days after percutaneous coronary intervention. *Circulation* 104:181–186, 2001
38. Trip MD, Cats VM, van Capelle FJ, Vreeken J: Platelet hyperreactivity and prognosis in survivors of myocardial infarction. *N Engl J Med* 322:1549–1554, 1990
39. Gurbel PA, Bliden KP, Guyer K, Cho PW, Zaman KA, Kreutz RP, Bassi Ak, Tantry US: Platelet reactivity in patients and recurrent events post-stenting: results of the PREPARE POST-STENTING study. *J Am Coll Cardiol* 46:1820–1826, 2005
40. Samara W, Bliden KP, Tantry US, Gurbel PA: The difference between clopidogrel responsiveness and posttreatment platelet reactivity. *Thromb Res* 115:89–94, 2005