Adenosine Diphosphate-Induced Single-Platelet Count Aggregation and Bleeding in Clopidogrel-Treated Patients Undergoing Coronary Artery Bypass Grafting

Magnus Dalén, MD,*† Jan van der Linden, MD, PhD,*† Manne Holm, MD,*† Gabriella Lindvall, MD, PhD,*† and Torbjörn Ivert, MD, PhD*†

Objectives: **To investigate the association between adenosine diphosphate (ADP)-induced platelet aggregation measured by single-platelet count testing and postoperative blood loss in clopidogrel-treated patients with acute coronary syndromes undergoing coronary artery bypass grafting (CABG).**

Design: **Prospective observational study.**

Setting: **Clinical study in one cardiac surgery center.**

Participants: **Eighty-eight patients treated with clopidogrel (300-600 mg loading dose followed by 75 mg daily) within 7 days before CABG.**

Interventions: **Platelet function was assessed preoperatively by single-platelet count ADP-induced platelet aggregation. Postoperative blood loss and transfusion requirements were recorded.**

Measurements and Main Results: **There was no significant association between ADP-induced platelet aggregation and blood loss 12 hours postoperatively (estimate −7.51; 95% confidence interval [CI]: −16.9-1.9; $p = 0.12$). ADP-induced platelet aggregation was associated significantly with the number of platelet concentrates administered within 24 hours after surgery (incidence rate ratio [IRR] 0.95; 95% CI: 0.92-0.98; $p < 0.01$), but not to the number of packed red blood cells (IRR 0.98; 95% CI: 0.95-1.01; $p = 0.14$).**

Conclusions: **Preoperative ADP-induced platelet aggregation measured by single-platelet count testing in clopidogrel-treated patients with acute coronary syndromes undergoing CABG was not associated with postoperative blood loss or packed red blood cells transfused, but was associated significantly with number of platelet concentrates administered during the initial 24 postoperative hours.**

KEY WORDS: coronary artery bypass, clopidogrel, platelet function tests, postoperative bleeding, platelets

PREOPERATIVE CLOPIDOGREL THERAPY is associated with increased postoperative blood loss, transfusion of blood products, rates of re-exploration for bleeding, and prolonged hospitalization in patients undergoing cardiac surgery.[1,2] Current guidelines recommend discontinuation of clopidogrel at least 5 days before coronary artery bypass grafting (CABG).[3–5] However, discontinuation of clopidogrel is associated with an increased risk of ischemic events and death while awaiting surgical revascularization.[1,2]

There is an interindividual variability in platelet response to clopidogrel as well as recovery of platelet function after clopidogrel discontinuation.[6–8] The degree of platelet inhibition in clopidogrel-treated patients accepted for CABG is, therefore, highly variable. Monitoring of platelet function in these patients may be used to optimize timing of surgery and to guide administration of blood transfusions.[9,10] Because the evidence of an association between platelet aggregation and blood loss after CABG is limited, individualized timing of surgery based on platelet function testing currently is not recommended.[10] Recent guidelines suggest that point-of-care testing of adenosine diphosphate (ADP)-induced platelet aggregation may be used to identify patients with high residual platelet reactivity who may not require a preoperative waiting period after clopidogrel discontinuation.[11] There are currently no recommendations regarding which platelet function test should be used in this setting.

In this study, the role of Plateletworks (Helena Laboratories, Beaumont, TX), a point-of-care platelet function test, in the preoperative assessment of bleeding risk in patients undergoing CABG was investigated. Plateletworks is based on single-platelet count, designed to measure residual platelet reactivity during antiplatelet therapy, and correlates well with light transmission aggregometry in measuring response to clopidogrel.[12] The ability of single-platelet count platelet aggregation testing to predict postoperative blood loss and administration of transfusions after CABG previously has not been studied thoroughly in clopidogrel-treated patients with acute coronary syndromes.

The primary objective of this study was to investigate the association between ADP-induced platelet aggregation measured by single-platelet count testing and postoperative blood loss in clopidogrel-treated patients with acute coronary syndromes undergoing CABG. A secondary aim was to investigate the association between ADP-induced platelet aggregation and transfusion of blood products.

METHODS

From June 2007 through May 2011, adult patients with an acute coronary syndrome undergoing primary isolated CABG with preoperative intake of clopidogrel within 7 days before surgery were studied prospectively. Patients with non-ST-elevation acute coronary syndromes (NSTE-ACS) or ST-elevation myocardial infarction (STEMI) were included. Patients treated with warfarin, with intake of $P2Y_{12}$-receptor inhibitors prasugrel or ticagrelor within 7 days, with glycoprotein IIb/IIIa inhibitors eptifibatide or tirofiban within 4 hours before surgery, or glycoprotein IIb/IIIa inhibitor abciximab within 12 hours before surgery were excluded. Type, dosage, and administration times of preoperative antiplatelet therapy were confirmed by contact with the referring hospital. Clopidogrel therapy (300-600 mg

*From the *Department of Cardiothoracic Surgery and Anesthesiology, Karolinska University Hospital; and †Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden.*

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Address reprint requests to Magnus Dalén, MD, Department of Cardiothoracic Surgery and Anesthesiology, Karolinska University Hospital, SE-171 76 Stockholm. E-mail: magnus.dalen@karolinska.se

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loading dose followed by 75 mg daily) was discontinued at the time the patient was accepted for surgery. Aspirin therapy was not withdrawn. The primary outcome measure was chest tube output at 12 hours after skin closure. Secondary outcome measures were transfusion of packed red blood cells and platelet concentrates within the first 24 hours postoperatively. The study was approved by the Regional Ethical Review Board in Stockholm.

A bolus dose of 2 g of tranexamic acid was given before skin incision followed by infusion of 1 g/hour during surgery. Aprotinin was not used. Surgery was performed with the aid of cardiopulmonary bypass after heparinization (400 IU/kg; activated coagulation time (ACT) maintained $\geq$480 seconds) and at a temperature of 34°C to 37°C. After weaning from cardiopulmonary bypass, heparin was neutralized with protamine at a 1:1 ratio. Chest tubes were placed in the mediastinum and opened pleural cavities.

After transfer to the cardiothoracic intensive care unit, chest tube output was registered hourly. The ACT was analyzed in case of bleeding more than 200 mL during the first postoperative hour or more than 100 mL/h after the first postoperative hour. An additional 50 mg of protamine were administered if the ACT remained more than 140 seconds. If excessive bleeding persisted, 1 unit of platelet concentrates (1 unit equals 300 mL of platelet concentrates obtained from 6 donors) was transfused and a coagulation status including platelet count, prothrombin time, activated partial thromboplastin time, fibrinogen, and antithrombin III was performed. Results from the laboratory tests guided administration of coagulation factor concentrates and plasma transfusions. Packed red blood cells were transfused if the postoperative hemoglobin level was less than 70 g/L and repeated if indicated by subsequent testing. Hemodynamic instability in combination with bleeding of more than 500 mL/h, or more than 300 mL/h in 2 consecutive hours, despite amendment of coagulation disorders, were indications for surgical re-exploration. Chest tubes were removed the day after surgery if bleeding was less than 30 mL/h for 3 consecutive hours.

Immediately before induction of anesthesia, blood for assessment of platelet function was drawn from the arterial catheter. Platelet aggregation was assessed using Plateletworks, which is a point-of-care platelet function assay based on whole blood single-platelet count after agonist-induced aggregation. The person performing the blood sampling and platelet aggregation measurements had received training by the manufacturer in managing the apparatus. One mL of fresh whole blood was added to each of 2 tubes; the tubes were primed with ethylenediaminetetraacetic acid (EDTA; 1.8 mg) and EDTA and ADP (final concentration 20 μmol/L), respectively. For each tube, platelet count was measured using a cell counter (ABX Micros 60, Horiba ABX Diagnostics, Holliston, MA) within 2 minutes from blood sampling. The EDTA sample served as baseline platelet count. The cell counter only detected nonaggregated platelets. Therefore, agonist-induced platelet aggregation decreased the platelet count. The percentage of nonaggregated platelets after addition of ADP was calculated by dividing the ADP sample platelet count with the EDTA sample platelet count multiplied by 100. The remaining proportion constituted the percentage of aggregated platelets. Because ADP was used as the agonist, this was an assessment of $P2Y_{12}$-receptor inhibition and, consequently, an assessment of residual platelet reactivity after intake of $P2Y_{12}$-receptor antagonist such as clopidogrel.

A generalized linear model was used for 12-hour chest tube output and ADP-induced platelet aggregation, adjusting for age. Assumptions about normality of the error distribution and linearity between the dependent and independent variables were checked. Poisson regressions were used for both number of platelet concentrates and packed red blood cells versus ADP-induced platelet aggregation. Both models were age-adjusted. Assumptions about equal mean and variance were good enough for the model with number of platelet concentrates. A Pearson scale parameter was used in the model for number of packed red blood cells. Statistical analyses were performed using SAS 9.2 (SAS Institute, Inc., Cary, NC).

RESULTS

All 88 enrolled patients were on continuous aspirin therapy, 75 mg daily. Seventeen patients were treated with fondaparinux within 24 hours before surgery. Patient characteristics and laboratory variables are presented in Table 1. Median duration between last dosage of clopidogrel and start of surgery was 48 hours and ranged between 5.5 and 168 hours.

Data regarding blood loss and transfusions are presented in Table 2. Blood loss 12 hours postoperatively ranged between 110 to 1,970 mL. During the initial 24 postoperative hours, packed red blood cells were administered to 50% of the patients and platelet concentrates to 30%. Five patients (6%) were re-explored for bleeding during the first 12 postoperative hours. A surgical source of bleeding was found in only one of these patients.

There was no significant association between ADP-induced platelet aggregation and blood loss 12 hours postoperatively (estimate –7.51; 95% confidence interval [CI]: –16.9-1.9; $p = 0.12$; Fig 1). Preoperative ADP-induced platelet aggregation was associated significantly with number of platelet concentrates administered within 24 hours after surgery (incidence rate ratio [IRR] 0.95; 95% CI: 0.92-0.98; $p < 0.01$; Fig 2). There was no association between ADP-induced platelet aggregation

Table 1. Patient Characteristics and Laboratory Variables

Characteristics	Total (n = 88)
Age, years	67.5 [62.0-73.3]
Female gender	17 (19%)
Body mass index, kg/m^2	26.7 [24.8-29.0]
Diabetes mellitus	23 (26%)
ST-elevation myocardial infarction	4 (5%)
Non-ST-elevation acute coronary syndrome	84 (95%)
Preoperative antiplatelet and anticoagulant therapy	
Duration between last dosage of clopidogrel and start of surgery, hours	48.0 [25.0-78.3]
Fondaparinux within 24 hours	17 (19%)
Preoperative laboratory data	
Hemoglobin, g/L	128 [117-138]
Hematocrit, %	37 [35-41]
Platelet count, cells/nL	214 [176-242]
Creatinine, g/L	83 [73-97]
Activated partial thromboplastin time, seconds	35 [30-38]
Prothrombin complex, international normalized ratio	1.0 [1.1-1.2]
ADP-induced platelet aggregation, %	92.3 [86.1-95.3]
Postoperative laboratory data, maximum	
Creatinine kinase MB, μg/L	10 [8-18]
Troponin T, ng/L	29 [18-72]
Aspartate transaminase, μkat/L	0.78 [0.60-1.47]
Creatinine, g/L	84 [46-116]
Cardiopulmonary bypass duration, minutes	64 [43-84]
Length of hospital stay, days	6.9 ± 3.1

NOTE. Data presented as median and interquartile range or number (%).

Abbreviations: ADP, adenosine diphosphate; MB, muscle and brain.