The Heart Surgery Forum #2004-1010
7 (3), 2004 [Epub April 2004]
doi:10.1532/HSF98.20041010

Online address: www.hsforum.com/vol7/issue3/2004-1010.html

Platelet Function Changes as Monitored by Cone and Plate(let) Analyzer during Beating Heart Surgery

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ABSTRACT

Background: Off-pump coronary artery bypass (OPCAB) is believed to reduce cardiopulmonary bypass (CPB)-related complications, including platelet damage. A hypercoagulable state instead of coagulopathy has been reported following OPCAB surgeries due to CPB. Whether platelet function is changed when the injurious effect of CPB is eliminated was investigated.

Methods: Platelet function was determined with the cone and plate(let) analyzer (CPA) method. The 2 parameters, average size (AS) and surface coverage (SC) of platelet aggregates, were measured with the CPA method to assess platelet aggregation and adhesion. These parameters were evaluated, and their values were compared at several stages of OPCAB surgery. The correlations of postoperative bleeding with platelet function at different stages of the surgery and with other factors, such as platelet count, hematocrit, and transfusions, were studied.

Results: Both AS and SC increased during several stages of the operation, and postoperative values (mean ± SD) were significantly higher than preoperative values (30.4 ± 8.1 μm^2 versus 23.3 ± 6.9 μm^2 for AS [P = .02] and 7.6% ± 3.6% versus 5.2% ± 1.8% for SC [P = .04]). The mean total bleeding volume was 875 ± 415 mL. Preoperative AS and SC were the only parameters significantly (P = .01) and linearly (r = 0.7) related to postoperative bleeding.

Conclusions: An increased platelet function, as determined by the CPA method, is found following OPCAB surgery. This phenomenon is probably at least partially responsible for the thrombogenic state after OPCAB surgery. Lack of platelet injury attributed to CPB may divert the system toward a more thrombogenic state. Preoperative platelet function, as evaluated by the CPA method, is an independent risk factor determining postoperative bleeding.

Received February 2, 2004; accepted February 3, 2004.

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INTRODUCTION

Off-pump coronary artery bypass (OPCAB) surgeries have been performed with increasing frequency in recent years. The elimination of cardiopulmonary bypass (CPB) from cardiac surgery has been suggested as a favorable solution for a myriad of CPB-related complications. Platelet dysfunction is a common finding during cardiac surgery with CPB [Collman 1990, Weerasinghe 1998]. The circulation of blood through the foreign surfaces of extracorporeal circulation results in platelet activation and a decrease in platelet count and function. The primary result of platelet dysfunction is bleeding in most cases and thrombosis in others. Along with causing coagulation disorders, a major activation of platelets during CPB releases multiple mediators with significant broad-spectrum systemic effects.

Indeed, analyses of outcomes after OPCAB operations have revealed a reduction in postoperative bleeding, which is a result of platelet dysfunction. Along with the decrease in blood loss, the need for blood and blood products has decreased [Ascione 2001, van Dijk 2001]. In reality, bleeding complications have been reduced significantly; on the other hand, a higher rate of thromboembolic complications has been reported for off-pump patients [Cartier 2001, Quigley 2002]. These complications are related to a hypercoagulable state occurring in the operations without CPB.

A hypercoagulable state has usually been reported for general surgery patients [Kaboli 2003, Kroegel 2003]. Patients who undergo coronary surgery are even more prone to thrombotic complications before the operation because a hypercoagulable state, which is seen as an increased generation of thrombin and tissue factor, exists in these patients [Falciani 1998].

The consequences of a hypercoagulable state after off-bypass surgery may be far worse than those of coagulopathy following on-CPB surgery. Thrombi generated in the coronary microcirculation and on the intimal surface of bypass conduits may have severe detrimental consequences.

Whether changes in platelet function are involved in the hypercoagulable state after OPCAB surgery has not been fully evaluated. We measured the changes occurring in platelet function during OPCAB surgery with the cone and plate(let) analyzer (CPA).

The CPA method has been described for the evaluation of platelet function. The CPA was designed in an attempt to establish a method that would test platelet function at close to actual physiological conditions. The test provides a useful tool for the quick identification of both congenital and acquired platelet defects, for testing the effect of antiplatelet drug therapy, and for detecting prothrombotic states with platelet hyperfunction.

The CPA method has proven to be a useful modality for the assessment of platelet function in various clinical conditions, including the prediction of bleeding due to thrombocytopenia after chemotherapy [Kenet 1998], monitoring treatment with antiplatelet drugs (glycoprotein IIb/IIIa) [Varon 1998, Shenkman 2001], and the evaluation of other hypercoagulation disorders [Shenkman 2003].

The purpose of this study was to determine the changes occurring in platelet function during cardiac surgery without CPB and the association between platelet function and postoperative bleeding.

MATERIALS AND METHODS

The study population consisted of 18 consecutive patients who underwent first-time OPCAB surgery. All patients had a standard anesthesia regimen, and perioperative care followed an established protocol that was the same for all patients. In all patients, left internal mammary artery and saphenous vein grafts were used as conduits. Following the harvest of the mammary artery, heparin was injected at a dose of 1.5 mg/kg, and anticoagulation status was monitored with the activated clotting time. The desired activated clotting time was a value greater than 200 seconds.

Patients with a known coagulation disorder not related to anticoagulant medications or with a known disorder of platelet function or platelet count were excluded from the study. Patients who were administered antiplatelet agents such as glycoprotein IIb/IIIa were also excluded.

Patients treated with anticoagulant medications for medical conditions such as atrial fibrillation had their anticoagulant medications discontinued at least 2 days before admission to the hospital. After patient admission, coagulation function was determined, and intravenous heparin treatment according to the patient's risk and tendency for thrombosis was started while the patient waited for surgery. In these cases, heparin treatment was discontinued at least 6 hours prior to surgery.

Patient demographic data and all perioperative factors with possible effects on postoperative bleeding were recorded.

All uses of medications with a possible effect on platelet function or count were included in the data. These medications included aspirin, calcium channel blockers, beta-blockers, statins, nonsteroidal anti-inflammatory drugs, and subcutaneous injections of low molecular weight heparin.

Blood samples were drawn at several stages of the operation: (1) before surgery during the induction of anesthesia; (2) immediately after midsternotomy; (3) 10 minutes after heparin injection; (4) 10 minutes after protamine injection; and (5) at the end of the operation.

A 5-mL volume of blood was drawn and stored in a citrated tube until the end of the operation. The complete blood count was performed with an automated Coulter counter at the same time as the CPA evaluation of platelet function.

Patient Follow-up

Patient postoperative care was in accord with a predetermined and standard protocol used for all patients in the intensive care unit. All parameters related to bleeding or thrombotic events were recorded. Postoperative bleeding was measured as the total amount of bleeding from the time of chest closure until the removal of chest tubes. Furthermore, the number of blood or blood product transfusions, any episodes of exceptional bleeding, and any need for chest reexploration for pericardial tamponade or major bleeding were recorded.

The Cone and Plate(let) Analyzer (CPA) for Platelet Function Evaluation

A 200-μL aliquot of whole blood in a 0.38% sodium citrate solution was placed in a polystyrene plate (Nunc Multidish 4UBEH; Nunc, Roskilde, Denmark), and a defined shear rate was applied for 2 minutes by means of a cone and plate device. Then, the objects on the plate were stained, and an image analyzer was used to measure the percentage of plate surface coverage (SC) and the average size (AS) of the stained objects. Under these test conditions, only platelets, but not other blood cells, adhere to the plate surface. When normal blood is analyzed, platelet deposition is a shear- and time-dependent process that reaches maximal levels of approximately 20% SC and 40 to 50 μm^2 AS within 2 minutes at a high shear rate (1800 per second). These 2 parameters are positively correlated with platelet count and hematocrit, demonstrating the important role of red blood cells in primary hemostasis. The AS and SC values define objective values for the platelet functions traditionally known as aggregation and adhesion.

Statistical Analysis

Data were analyzed at both the univariate and multivariate levels. The collected data were entered into a statistical database as either continuous or categorical variables for comparative statistical analysis. Data are expressed as the mean ± SD and range values where applicable. The univariate analyses included the Student *t* test for the comparison of 2 groups of quantitative variables, the chi-square test for the assessment of association between 2 categorical variables, and the Pearson correlation coefficient for the calculation of correlation between 2 quantitative variables. The potential influence of different variables on postoperative bleeding amounts was evaluated by a multivariate general linear model. A *P* value of .05 or less was considered statistically significant.

RESULTS

The mean age of the patients was 62 ± 11.3 years. The male-female ratio was 15:3.

There was no perioperative mortality. No patient underwent reexploration for bleeding, and no patient received

Table 1. Preoperative and Operative Data*

Age, y	62.0 ± 11.3 (40-79)
M/F sex, n	15/3
Urgent operation, n	3
Aspirin, n	4
Calcium blocker, n	3
Beta-blocker, n	8
Statin, n	7
NSAID, n	0
LMWH, n	0

*Age is expressed as the mean ± SD (range). NSAID indicates nonsteroidal anti-inflammatory drug; LMWH, low molecular weight heparin.

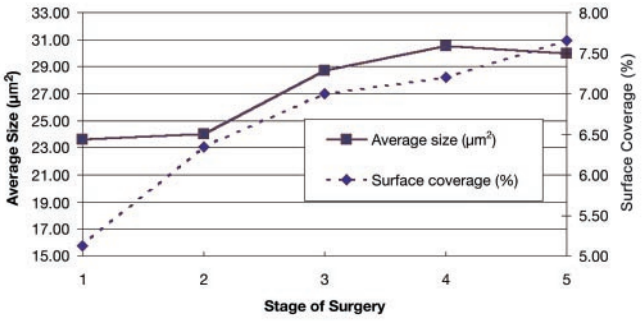
blood or blood product (including platelets) transfusion during the surgery. The preoperative and demographic data are summarized in Table 1.

Platelet Function during Surgery

Average Size. The preoperative AS was 23.3 ± 6.9 μm^2. The AS increased until the end of the operation, but the changes between the stages were not significant. The postoperative AS was significantly higher than the preoperative AS (30.4 ± 8.1 μm^2 compared with 23.3 ± 6.9 μm^2; $P = .02$) (Figure).

Surface Coverage. The SC increased at each stage of the operation until the end of the operation. Although the changes between successive stages were not significant, the SC at the end of the operation was significantly higher than the preoperative value (7.6% ± 3.6% versus 5.2% ± 1.8%; $P = .04$) (Figure).

Platelet Count. The mean platelet count before the operation was 185 ± 50 × $10^3/\mu L$. The platelet count decreased significantly to 146 ± 42 × $10^3/\mu L$ with the administration of heparin in stage 3, and then a slight recovery in the count ensued until the end of the operation. The platelet count at the end of the operation was still lower than the preoperative count (153 ± 38 × $10^3/\mu L$ versus 185 ± 50 × $10^3/\mu L$; $P = .04$).



Changes in platelet average size and surface coverage during the surgery.

Hematocrit

The preoperative hematocrit was 30.1% ± 5.1%. The hematocrit stayed stable during the several stages of the operation, and the postoperative hematocrit was similar to the preoperative measurement.

Transfusions

Blood transfusions included units of packed red cells and whole blood. Of the 18 patients enrolled in the study, 6 patients had transfusions during the postoperative course. Packed red cells were transfused in 3 patients, and whole blood was transfused in 4 patients.

The mean number of packed cell units transfused was 0.3 ± 0.4 per patient, and the mean number of whole blood units transfused was 0.2 ± 0.4 per patient.

Postoperative Bleeding

The mean postoperative bleeding volume was 875 ± 415 mL. The correlation between relevant factors and bleeding was assessed to determine the circumstantial relationship between the dependent factors.

Bleeding and AS. A significant negative correlation was found between preoperative AS and postoperative bleeding ($r = –0.7$; $P = .01$). Postoperative AS also showed a good correlation with postoperative bleeding ($r = –0.5$; $P = .05$).

Bleeding and SC. A significant negative correlation was also found between preoperative SC and postoperative bleeding ($r = –0.7$; $P = .009$). The postoperative SC had no correlation with postoperative bleeding ($r = –0.3$; $P = .2$).

Bleeding and Platelet Count. The correlation between postoperative bleeding and preoperative platelet count was not significant ($r = –0.3$; $P = .2$). A borderline correlation was seen between postoperative bleeding and platelet count in stage 8 of the surgery ($r = –0.4$; $P = .09$).

The univariate analysis model also included other parameters, such as preoperative medications, patient age and urgency of operation, preoperative and postoperative platelet count, and blood transfusions. Except for the preoperative platelet function variables AS and SC described earlier, the univariate analyses revealed no significant association between postoperative bleeding and any other perioperative factor incorporated in the study as a variable.

Multivariate Analysis

The relationship between all of the parameters assessed in the univariate analysis and bleeding was evaluated in the multivariate analysis model. These parameters were always entered initially into the model as independent variables (some were considered to be confounding variables, and others were considered risk factors). According to the multivariate model, postoperative bleeding was found to be significantly ($P = .01$) and linearly ($r = 0.7$) dependent on preoperative platelet function (AS and SC); none of the other independent variables were found to be statistically significant.

Bleeding Population Subgroups

A statistical analysis was done by dividing the postoperative bleeding patient group into 2 subgroups, a minor bleed-

Table 2. Comparison of Bleeding Groups*

Variable	Minor Bleeding (<800 mL) (n = 8)	Major Bleeding (>800 mL) (n = 10)	P
Bleeding, mL	517 ± 228	1168 ± 280	.0001
Average size, μm^2†	28.1 ± 7.8	19.7 ± 3.3	.02
Surface coverage, %†	6.4 ± 1.4	4.2 ± 1.3	.02
Platelet count, $\times 10^3/\mu L$†	207 ± 35	169 ± 55	.1
Hematocrit, %†	32.3 ± 4.5	28.5 ± 5.2	.1

*Data are expressed as the mean ± SD.
†Stage 1 of operation (before surgery).

ing group (less than 800 mL) and a major bleeding group (more than 800 mL).

The correlation analysis of these subgroups and platelet function as measured by AS and SC revealed interesting findings (Table 2). A significant negative association was seen between preoperative AS and bleeding subgroups. Patients with major postoperative bleeding had the lowest preoperative AS values (19.7 ± 3.3 μm^2) and patients with minor bleeding had the highest preoperative AS values (28.1 ± 7.8 μm^2). Similar findings were also noted in the association between preoperative SC and postoperative bleeding, with the minor and major bleeding groups having SC of 6.4% ± 1.4% and 4.2% ± 1.3%, respectively.

The 2 subgroups had similar demographics, and the preoperative data for these groups were not different with respect to bleeding-related factors.

These findings are of major importance in the use of preoperative factors to predict postoperative bleeding. When the measured AS is less than 20 μm^2 or the SC is less than 5%, the patient generally has a considerable risk for bleeding a large volume.

Postoperative Thromboembolic Events

The perioperative platelet function studies with CPA revealed no case of prothrombotic measures for either AS or SC. In the short-term follow-up period of the postoperative hospital stay, no cases of thromboembolic events were recorded in any patient.

DISCUSSION

Cardiac surgery is a procedure with a major deleterious influence on multiple systems. Almost the whole of homeostatic balance is affected by cardiac surgery [Collman 1990, Wan 1997].

Normal platelet function is essential following major procedures such as cardiac surgery. During the nonphysiological process of traditional cardiac surgery with extracorporeal circulation, platelets undergo extensive transformation such as activation and degranulation, and the consequences can be, apart from the systemic implications, coagulation disorders such as bleeding, thrombosis, and restenosis [Chandrasekar 2000]. Prediction of such consequences is of great importance because these sequelae are among those that determine the prognosis of cardiac surgery and that directly relate to mortality and morbidity [Edmunds 1996].

In this study, platelet function during various stages of cardiac surgery without CPB was determined by the CPA method, and the relationship between postoperative bleeding and platelet function and other factors was investigated.

Initially, it would seem that the elimination of CPB would remove the coagulation disorders due to platelet injury, which are manifested as postoperative bleeding. On the other hand, surgery without CPB has been reported to have a higher incidence of thrombotic complications [Cartier 2001]. A gradual, stepwise increase in platelet function during the several stages of operation, as seen in this study, may be due to or at least be a promoter for a thrombogenic state that has been described in OPCAB surgeries. Although the evaluation of other coagulation factors that have a fundamental role in the hemostasis process was not included in this study, a 30% increase in AS and a 45% increase in SC mean a corresponding augmentation of platelet aggregation and adhesion, the consequence of which may be a thrombogenic state in OPCAB surgery and not the hematogenic one seen when CPB has been administered.

Our study shows a clear tendency for the aggregation and adhesion of platelets at the end of OPCAB surgeries. By simulating the physiological conditions of platelets with CPA, we are able to conclude that platelet hyperfunction plays a role in the thrombogenic state after OPCAB surgeries, even though other studies have not found such a relationship. The changes in the coagulation profiles after OPCAB surgery reported by Mariani and colleagues have been attributed mainly to the coagulation system and not to platelets, because the platelet count and platelet activation remained at preoperative levels after the procedure [Mariani 1999]. This finding may have therapeutic implications that redirect a prophylactic treatment for the thrombotic state to focus instead on antiplatelet therapy rather than on anticoagulation therapy.

The other important finding of this study is the relationship between preoperative platelet function and postoperative bleeding. The importance of platelet function evaluation in the preoperative period and the involvement of platelet function in postoperative bleeding has attracted attention through the years toward the use of platelet function evaluation to predict bleeding. A French group examined platelet function with a high shear stress method (PFA 100) in patients who underwent coronary artery bypass grafting. No correlation was found between the calculated postoperative bleeding and platelet function determined before and after surgery by this method [Lasne 2000]. In that study, postoperative bleeding was calculated and not measured.

Dividing the patients into 2 bleeding subgroups in our study revealed a significant relationship between bleeding and preoperative platelet function. This finding is of major importance for the postoperative management of patients in the intensive care unit. The important finding of our study was that patients with a preoperative AS of less than 20 μm^2 or an SC of less than 5% had postoperative bleeding of approximately 1000 mL, whereas patients with the same findings but higher AS and SC values had only approximately 500 mL of blood loss after surgery. Apart from the need for

more transfusions and the inherent risk of viral infection related to transfusion, a bleeding volume of 1000 mL in cardiac surgery is of hemodynamic significance with the risk of cardiac tamponade and a decrease in graft flow becoming a more actual threat.

The predictive value of these observations is in identifying those individuals who, regardless of other factors, will unexpectedly have more bleeding due to preoperative platelet dysfunction. The possibility of preoperative platelet dysfunction is not uncommon and is a sporadic finding nowadays. A significant proportion of patients are treated with medications that affect platelet function to some degree. In an era when treatment with glycoprotein IIb/IIIa antagonists is becoming increasingly widespread, the evaluation of platelet function is of foremost importance. The efficiency of platelet function determination in patients treated with these agents and who have undergone carotid artery stenting has been evaluated [Coller 1998, Shenkman 2001].

In daily practice, a substantial number of patients arrive for coronary surgery a short time after the administration of the aforementioned drugs. The flip side of a high treatment efficacy is the possible risk of bleeding, especially in the case of a required surgery. A surgical procedure in these patients is a challenge for the surgical and intensive care teams because it demands special management techniques [Despotis 1999, Levy 2000]. On the other hand, the responses to such treatment are not homogeneous, and the assessment of platelet inhibition potency and the need to monitor treatment with these antagonists are of great importance [Lasne 2000].

The small subgroup of patients with preoperative platelet dysfunction would not be detected in routine tests unless a major surgery revealed its clinical expression as a bleeding diathesis. However, identification of this problem will enable us to adopt special measures to diminish any additional injury to platelets during a surgery and to treat the patient before the clinical manifestations of platelet malfunction.

In conclusion, an increase in the aggregation and adhesion of platelets during OPCAB surgery as evaluated by the CPA method has been noted. A hyperfunctional state of platelets after OPCAB surgery is probably at least a partial cause of the thrombogenic state in these patients. Preoperative platelet dysfunction has been found to be the most important factor determining postoperative bleeding.

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