



## Regular Article

# Reductions in platelet contractile force correlate with duration of cardiopulmonary bypass and blood loss in patients undergoing cardiac surgery<sup>☆</sup>

Philip E. Greilich<sup>a,\*</sup>, Chad F. Brouse<sup>a</sup>, Joseph Beckham<sup>a</sup>, Michael E. Jessen<sup>b</sup>,  
Erika J. Martin<sup>c</sup>, Marcus E. Carr<sup>c</sup>

<sup>a</sup>Department of Anesthesiology and Pain Management, University of Texas Southwestern Medical Center—Dallas, Veterans Affairs North Texas Health Care System, Dallas, TX, USA

<sup>b</sup>Department of Cardiovascular and Thoracic Surgery, University of Texas Southwestern Medical Center—Dallas, Veterans Affairs North Texas Health Care System, Dallas, TX, USA

<sup>c</sup>Departments of Internal Medicine and Pathology, Central Virginia Coagulation Disorders Center, Virginia Commonwealth University, Richmond, VA, USA

Received 2 April 2002; accepted 2 April 2002

Accepting Editor: C.-E. Dempfle

## Abstract

Blood loss secondary to platelet dysfunction is known to be increased when the duration of cardiopulmonary bypass (CPB) is prolonged. The ability to correlate alterations in platelet function with the duration of bypass and early postoperative blood loss, however, has remained elusive. Platelet contractile force, a novel measure of platelet-mediated clot retraction, is known to be reduced following cardiac surgery and blockade of platelet adhesion receptors. The aim of this study was to determine if alterations in platelet contractile force (measured using whole blood) correlated with the duration of CPB and early postoperative blood loss. Thirty patients were entered into a study designed to measure platelet function before, during, and after CPB. Platelet aggregometry and surface expression of CD42b and CD61 were also measured (using whole blood) in a subset of subjects ( $n = 10$ ) to further characterize the intrinsic structural and functional defects induced by CPB. Reductions in platelet contractile force had a significant correlation with duration of CPB ( $r = 0.564$ ;  $P = 0.002$ ) and early blood loss ( $r = 0.545$ ;  $P = 0.003$ ). Although decreases in platelet contractile force and aggregation both correlated with CPB time in the smaller subset of patients tested, only platelet contractile force correlated with decreases in CD42b, CD61 and blood loss. The results of this study suggest that prolongation of CPB is related to increasing degrees of platelet dysfunction and that reductions in platelet contractile force are related to decreases in platelet adhesion receptors and early postoperative blood loss. © 2002 Elsevier Science Ltd. All rights reserved.

**Keywords:** Platelet contractile force; Platelet aggregation; Platelet adhesion receptors; Cardiopulmonary bypass; Hemorrhage

## 1. Introduction

Platelet dysfunction is believed to be the primary cause of excessive microvascular bleeding following cardiopul-

monary bypass (CPB) surgery [1–3]. While an increased bleeding time has long been considered a hallmark of platelet dysfunction [1,2], measured decreases in platelet aggregation [1,2,4–6] and platelet glycoprotein adhesive receptors [7–11] have also been shown in some studies to be associated with blood loss. Platelet contractile force is a novel measurement of platelet function that quantitatively assesses platelet-mediated clot retraction [12]. Platelet contractile force has been shown to be reduced when platelet aggregation is decreased secondary to uremia [13], following cardiac surgery [14] and platelet adhesion receptor blockade [15–17]. When measured in platelet-rich plasma, platelet contractile force has also been shown to correlate with blood loss following CPB surgery [14].

*Abbreviations:* CPB, Cardiopulmonary bypass; MFI, Mean fluorescence intensity.

<sup>☆</sup> Presented at the 43rd Annual meeting of the American Society of Hematology, December 7–11, 2001, Orlando, Florida.

\* Corresponding author. Anesthesiology and Pain Management Service (112A), Veterans Affairs North Texas Health Care System, 4500 S. Lancaster Road, Dallas, TX 75216, USA. Tel.: +1-214-857-1818; fax: +1-214-857-1867.

E-mail address: philip.greilich@email.swmed.edu (P.E. Greilich).

Platelet dysfunction acquired during CPB is undoubtedly multifactorial. The intrinsic platelet defects are considered to be a combination of both structural and functional abnormalities. The functional defects are primarily attributed to CPB-induced platelet activation [1,2,18] and possibly direct effects of heparin [2,19]. Structural alterations involving key surface adhesion [7–9] and thrombin receptors [20,21] also contribute to the genesis of this acquired platelet function abnormality. Unlike many platelet bioassays, which may characterize only a single aspect of platelet structure or function, the dependence of the platelet contractile force measurement on platelet aggregation, adhesion receptor function and thrombin activation may permit it to detect multiple intrinsic platelet defects following CPB surgery.

If the primary etiology of the intrinsic platelet defect or “battered platelet syndrome” is contact with the nonendothelialized membrane oxygenator of the bypass circuit, then the degree of dysfunction should correlate with the length of time that platelets are exposed to such an insult. In order to be clinically relevant, this measure of platelet function should also correlate with blood loss following CPB surgery. The primary aim of this study was to determine if alterations in platelet contractile force, measured in whole blood, correlated with the duration of CPB and early postoperative blood loss. A secondary aim of the study was to determine if whole-blood platelet aggregation also correlated with CPB time and if observed changes in the *ex vivo* measurements of platelet function (platelet contractile force and aggregation) correlated with altered expression of two key platelet adhesion receptors (CD61, CD42b).

## 2. Materials and methods

### 2.1. Patient selection

Following institutional review board approval by the Dallas Veterans Affairs Medical Center, patients scheduled for elective, cardiac surgery requiring CPB were enrolled in the study. Patients were excluded if they received corticosteroids, dipyridamole, or anticoagulants or had documented platelet or coagulation abnormalities, or had treatment with thrombolytic therapy within 5 days of surgery. Other exclusion criteria included a creatinine  $>2.0$  mg/dl, ejection fraction  $<30\%$ , and a history of adverse reaction to  $\epsilon$ -aminocaproic acid. Patients were not excluded from the study if they were receiving salicylates, nonsteroidal anti-inflammatory drugs, or heparin preoperatively.

### 2.2. Sample collection

Arterial blood samples were collected: (1) before induction (baseline), (2) during CPB (normothermic CPB, following cross-clamp removal), and (3) post-CPB (within 2 h after the termination of CPB). Blood was drawn from the radial arterial line into a polypropylene syringe and was

transferred immediately into a sterile 3.2% Na-citrate-buffered Becton Dickinson brand Vacutainer. Heparinase I (IBEX, Montreal, Quebec, Canada) was added to whole-blood samples (1.5  $\mu\text{g/ml}$ , final concentration) to remove residual heparin prior to performing the platelet contractile force assay.

### 2.3. Technique of operation

Preoperative medications, including  $\beta$ -blockers, nitrates, and antiarrhythmics, were continued until surgery. After sedation with midazolam 2–5 mg, intravenous access, a radial arterial cannula, and routine monitors were placed. Following induction with etomidate, 0.3 mg/kg, fentanyl, 5–10  $\mu\text{g/kg}$ , and rocuronium 1 mg/kg, a pulmonary artery catheter was inserted via the right internal jugular vein. Anesthesia was maintained with isoflurane, 0.2–1.0% inhaled, and fentanyl, 25–50  $\mu\text{g/kg}$ . A propofol infusion, 25–50  $\mu\text{g/kg/min}$  was started at the beginning of rewarming during CPB and continued into the postoperative period for sedation.

All the procedures were performed by one of three surgeons using a standardized technique for coronary revascularization, valve repair/replacement and myocardial protection. Extracorporeal circulation was performed using a membrane oxygenator (Gish, Irvine, CA) with nonpulsatile flow using a centrifugal pump (Biomedicus, Eden Prairie, MN). The extracorporeal circulation circuit was primed with 2 L of lactated Ringer's solution, 100 cc of 25% albumin, 44.6 meq of sodium bicarbonate and 50 g of mannitol. Perfusion was done at moderate systemic hypothermia (28–32 °C) and myocardial protection was achieved using antegrade and retrograde sanguineous (4:1, blood:cardioplegia) cardioplegia administered every 20 min. Perfusion flow rates were maintained at 2 L/min/mm<sup>2</sup> during hypothermia and at 2.5 L/min/mm<sup>2</sup> during normothermia.

Anticoagulation was achieved with bovine heparin and monitored to initially achieve a kaolin activated clotting time (ACT) level of 480 s and then the heparin level was maintained using the Hepcon (Medtronic, Parker, CO) system. Temperature was monitored via a bladder probe and separation of bypass was initiated at 36.5 °C. Reversal and the presence of residual heparin were monitored with a protamine titration protocol.

Blood was collected from the surgical field via pump suction during full anticoagulation and returned to the patient intraoperatively. Mediastinal chest drainage was not reinfused. Packed red blood cells were transfused when the hemoglobin level fell below 8.0 g/dl and platelet concentrates were given for platelet counts  $<70,000/\mu\text{l}$  in patients with clinical evidence of microvascular bleeding.

### 2.4. Measurement of platelet contractile force

Determination of platelet contractile force was performed on the Hemodyne hemostasis analyzer model RM-2 (Hemo-

dyne, Richmond, VA) as previously described by our laboratory [16]. Physiologic levels of thrombin (1 U/ml) and calcium chloride (5–10 mM) were added to initiate clot formation at 37 °C. Final platelet concentration after dilution was 90% of the actual concentration at the time of collection. Each measurement lasted 20 min, and each sample was tested in duplicate.

### 2.5. Measurement of platelet aggregation

Platelet aggregation studies were performed using a dual-channel impedance aggregometer (Chrono-Log, Havertown, PA) as previously described [5]. The platelet count was determined using Coulter T-540 whole-blood counter (Coulter, Hialeah, FL) and the platelet count was corrected to 75,000 platelets per  $\mu\text{l}$  by dilution with physiological saline solution. Collagen (Chrono-Log, 10  $\mu\text{g}/\text{ml}$ , final) was used as an agonist and was added at time zero.

### 2.6. Flow cytometric analysis

Platelet adhesion receptor expression was determined by flow cytometry. Duplicate aliquots of whole blood (5  $\mu\text{l}$ ) were placed into two 12  $\times$  75 polypropylene falcon tubes containing 20  $\mu\text{l}$  each of CD61 FITC (Becton Dickinson clone RUU-PL 7F12) and CD42b PE (Pharmingen clone HIP1), and either 10  $\mu\text{l}$  of TRAP (2 mM) or 10  $\mu\text{l}$  of Tyrode's buffer. The tubes were then incubated at room temperature for 20 min in the dark. After incubation, the samples were fixed in 1% paraformaldehyde and stored at 4 °C until analyzed later on the day of collection.

Samples were analyzed using a FACstar Plus flow cytometer (Becton and Dickinson Immunocytometry Systems). In each experiment, one sample was stained with

Table 2  
Coagulation and platelet function data

Variable	Baseline	CPB	Post CPB
Platelet contractile force ( $\times 10^3$ , dynes)	11.8 $\pm$ 3.31	8.82 $\pm$ 2.63	8.48 $\pm$ 2.56
Platelet aggregation	19.6 $\pm$ 10.5	11.1 $\pm$ 6.91	14.7 $\pm$ 8.05
Platelet counts ( $\times 10^3/\mu\text{l}$ )	174 $\pm$ 50.4	115 $\pm$ 35.3	107 $\pm$ 29.3
Fibrinogen (mg/dl)	413 $\pm$ 125	214 $\pm$ 75.6	208 $\pm$ 95.5
Hematocrit (%)	37.1 $\pm$ 3.66	23.9 $\pm$ 2.87	26.3 $\pm$ 3.42

Values shown are mean  $\pm$  standard deviation.

FITC- and PE-conjugated, irrelevant isotypes, which served as the negative control. Platelets were gated using forward and side scatter and the purity of the gated cells were assessed using antibodies directed against either CD42b or CD61. The mean fluorescence intensity (MFI) was used to quantify platelet CD42b and CD61 expression. Only activated CD61 and unactivated CD42b were analyzed as we believe this represents the most likely physiologic state when each of these receptors engage their respective primary ligands.

### 2.7. Statistical analysis

Statistical analysis was performed with GraphPad Prism Software (GraphPad Prism Software, San Diego, CA). Descriptive statistics were expressed as mean  $\pm$  standard deviation unless otherwise stated. A linear regression analysis was used to examine relationships between data. A value of  $P < .05$  was considered statistically significant.

## 3. Results

### 3.1. Patient and surgical data

Following written informed consent, a total of 30 subjects were enrolled in the study. Complete data were obtained from only 28 of the subjects that consented, as one subject expired before the post-CPB data could be collected and another had an incomplete data set due to unavoidable logistical reasons. For patients' demographic and surgical data see Table 1. The actual platelet counts, fibrinogen concentrations, platelet contractile force and platelet aggregation values are shown in Table 2.

### 3.2. Platelet functional assays and CPB time

The correlation between decreases in platelet contractile force and CPB time is illustrated in Fig. 1. The decrease in platelet contractile force (expressed as a percentage of baseline) measured post-CPB significantly correlated ( $r = 0.564$ ;  $P = 0.002$ ) with the duration of CPB. Platelet aggregometry was measured in a subset of 10 of the 28

Table 1  
Patient and surgical demographics

Variable	
No. of patients	28
Age (years)	63.0 $\pm$ 8.76
Body weight (kg)	94.9 $\pm$ 25.4
Body surface area ( $\text{m}^2$ )	2.08 $\pm$ 0.18
Ejection fraction (%)	55 $\pm$ 15
Aspirin/NSAID	25/28 (89%)
Procedure	
No. of CABG only	18
No. of valve only	5
No. of valve + CABG	5
CPB time (min)	
Minimum	65
Median	130
Maximum	251
Cross-clamp time (min)	88.2 $\pm$ 33.0
Total heparin ( $\times 10^2$ IU)	430 $\pm$ 86.1
Total protamine (mg)	342 $\pm$ 141
4 h blood loss (ml)	397 $\pm$ 357

Values shown are mean  $\pm$  standard deviation, unless indicated otherwise.

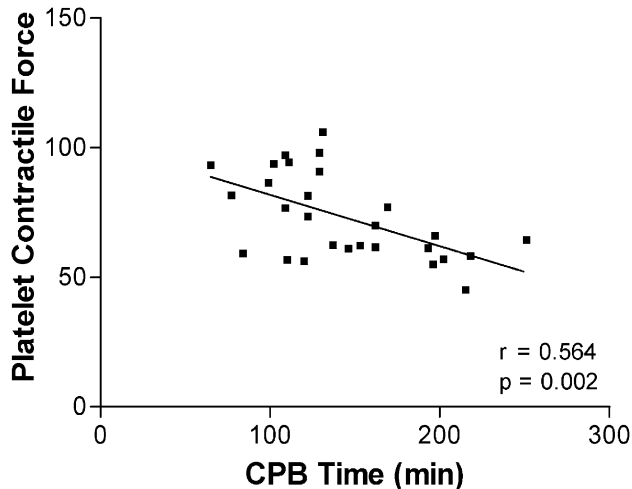


Fig. 1. Correlation between platelet contractile force (PCF) and cardiopulmonary bypass (CPB) time. PCF was measured in whole blood following CPB and is expressed as a percentage of the baseline (preinduction) value.

subjects and was also found to significantly correlate ( $r=0.744$ ;  $P=0.014$ ) with the duration of CPB. The correlation between platelet contractile force and CPB time in this same subset of patients was also significant ( $r=0.791$ ;  $P=0.006$ ). There was no correlation between decreases in platelet counts and duration CPB or platelet contractile force.

### 3.3. Platelet functional assay and early postoperative blood loss

The relationship between platelet contractile force and early (first 4 hours following CPB) blood loss (mediastinal chest tube drainage) is illustrated in Fig. 2. The decrease in platelet contractile force (expressed as a percentage of

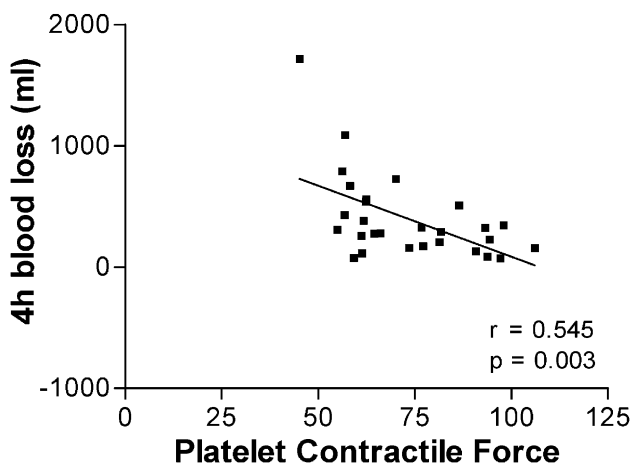


Fig. 2. Correlation between platelet contractile force (PCF) following cardiopulmonary bypass (CPB) and blood loss. PCF is expressed as a percentage of the baseline (preinduction) value and blood loss is expressed as the volume of chest tube drainage during the first 4 h after mediastinal chest tube placement.

baseline) post-CPB correlated ( $r=0.545$ ;  $P=0.003$ ) with 4 h blood loss. Although the decreases in platelet contractile force also correlated ( $r=0.673$ ;  $P=0.033$ ) with early postoperative blood loss for the subset of patients in which platelet aggregation was determined, decreases in aggregation did not correlate with blood loss ( $P=0.8$ ).

### 3.4. Platelet functional assays and loss of adhesion receptor following CPB

Platelet adhesion receptor (CD61, CD42b) analysis was performed on 10 of the 28 subjects analyzed in this study. These subjects were the same 10 subjects that also had platelet aggregation assays performed. The data in Fig. 3a and b demonstrate that decreases in platelet contractile force correlate with activated CD61 receptor expression (Fig. 3a;  $r=0.744$ ;  $P=0.014$ ) and CD42b (Fig. 3b.  $r=0.697$ ;  $P=0.025$ ) when measured following CPB. No correlation

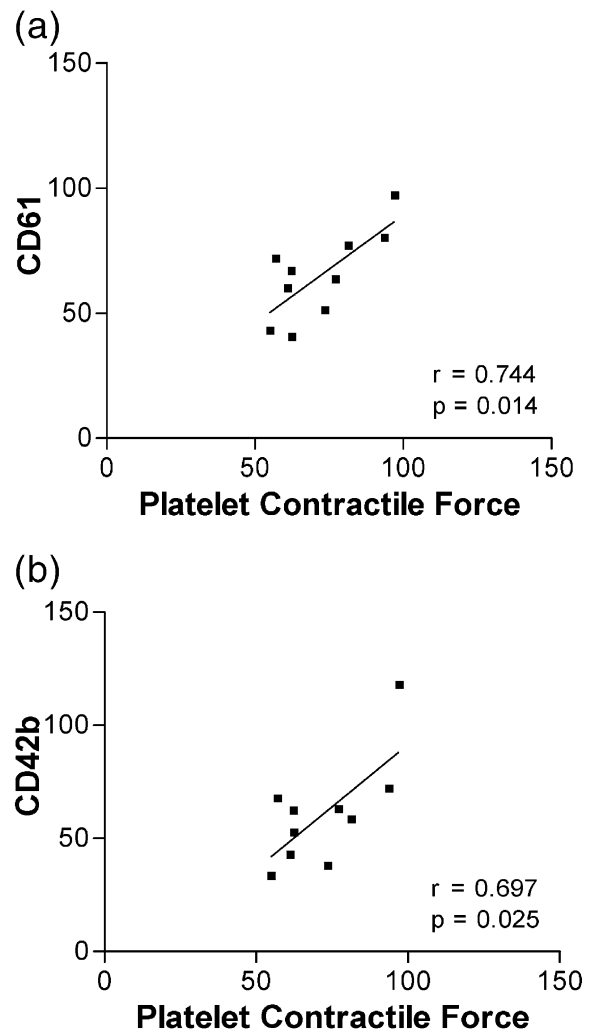


Fig. 3. Correlation between platelet contractile force (PCF) and surface expression of activated CD61 (a) and CD42b (b) following cardiopulmonary bypass (CPB). PCF is expressed as a percentage of the baseline (preinduction value) and activated CD61 and CD42b are expressed as mean fluorescence intensity (MFI).

could be found between decreases in platelet aggregation and decreases in CD61 ( $P=0.7$ ) and CD42b ( $P=0.7$ ).

#### 4. Discussion

This investigation tests the hypothesis that the duration of CPB influences the *degree* of platelet dysfunction following cardiac surgery. Our results demonstrate that decreases in platelet contractile force and aggregation correlate with duration of CPB when measured using whole blood. Unlike aggregometry, platelet contractile force also correlates with early postoperative blood loss. A direct correlation was also found between decreases in platelet contractile force and the measured MFI of two platelet adhesion receptors (CD42 and CD61). Collectively, these results suggest that the measurement of the platelet contractile force is reflective of intrinsic structural and functional platelet defects induced by CPB, and furthermore that the degree of alteration in platelet contractile force correlates with the degree of blood loss during the early postoperative period.

Although platelet dysfunction is considered to be the primary cause for microvascular bleeding following CPB [1–3], the ability to experimentally characterize the relationship between intrinsic platelet defects and blood loss continues to be a challenge. Some authors contend the defect is not primarily intrinsic, but rather an extrinsic defect possibly due to insufficient platelet agonist at the site of clot formation [2]. The ability of the platelet contractile force to detect significant intrinsic platelet dysfunction using whole blood in this study reinforces previous work using platelet-rich plasma [14]. Similar to platelet aggregometry, time (CPB duration)-dependent decreases in contractile force suggest that prolonged exposure to the CPB circuit clearly contributes to the degree of this intrinsic defect. Additionally, the correlation of platelet contractile force (both platelet-rich plasma and whole blood), with early postoperative blood loss suggests that the platelet's ability to initiate and sustain a contractile force plays a role in minimizing microvascular bleeding following CPB.

The platelet contractile force assay measures the force generated during platelet-mediated clot retraction [12]. The quantitative measure of platelet contractile force depends not only on the functional integrity of platelet cytoplasmic proteins (actin–myosin), but also on the expression of conformationally active structural adhesive receptors [13]. Optimal platelet activation ensures both the extension of the actin–myosin mediated pseudopodia and the conformational changes needed for high-affinity ligand binding by CD61 and CD42b [22,23]. Shortly after CD61-mediated fibrinogen binding, contraction of actin filaments results in retraction of the platelet pseudopodia toward the platelet central mass. The subsequent development of contractile force and the consequent retraction process produces tension within the clot structure leading to a measurable increase in elastic modulus and enhanced clot rigidity [23]. Whether

due to thrombocytopenia [12,23] congenital defects [24], disease state [14,17] or pharmacological blockade [13,15,25], *in vivo* impairment of any one of the mechanisms contributing to platelet-mediated clot retraction could potentially lead to increased blood loss. The correlation of platelet contractile force and blood loss in this study is most likely due to this assay's ability to detect *multiple* mild-to-moderate structural and functional defects when measured with physiologic concentrations of calcium and thrombin in whole blood.

Surface expression of platelet CD42b (GP1b) and CD61 (GPIIb/IIIa) are critical to effective platelet adhesion and aggregation [7–9]. Although the primary ligand for CD42b and CD61 are von Willebrand factor and fibrinogen, respectively, crossover is known to occur when the receptors' primary ligand concentrations are low [24,26]. Alterations in platelet adhesive receptors following CPB represent a potential structural link to decreases in intrinsic platelet function like platelet-mediated clot retraction. Several studies have demonstrated that platelet activation during CPB can lead to significant reductions in CD42b and CD61 expression [4,7–11,27,28]. If combined with significant reductions in these receptors' ligand concentrations (by hemodilution or consumptive processes), a platelet's ability to translate its contractile force to the clot's surface could be impaired, thus leading to a decrease in the platelet contractile force measurement. Indeed, alterations in platelet adhesive receptor function have been shown to lead to excessive blood loss following CPB surgery [3,29]. Although our data suggest a clinically relevant link between alterations in platelet contractile force and CD61 and CD42b, it remains unclear whether it is due to decreases in: (1) receptor density, (2) ligand concentration, (3) functional integrity of the receptor, and/or (4) effectiveness of actin–myosin contractile activity.

Despite the use of whole-blood samples for measuring platelet aggregation and contractile force, decreases in platelet aggregation following CPB failed to correlate with decreased adhesion receptor expression under the experimental conditions used in this study. The difference in the presence or absence of extracellular calcium and type of agonist used for each of the assays may have contributed to our findings. Previous studies have shown an association between blood loss and collagen-induced platelet aggregation and for this reason, collagen was added to the citrated whole blood used for this assay [5]. In contrast, platelet contractile force is a thrombin-dependent process that is either delayed or reduced when either prothrombin conversion is deficient or thrombin activity is inhibited [30]. As such, physiologic concentrations of calcium and thrombin are used to ensure the measurement of peak platelet contractile force [12]. Perhaps the *ex vivo* addition of optimal concentrations of calcium and thrombin to the platelet contractile force assay more closely reflects the patient's intrinsic platelet function and thus the correlation with the loss of adhesion receptors was more evident. Alternatively, a

direct antiplatelet effect of heparin on platelet aggregation could have influenced these measurements as *Heparinase I* was only added to whole blood samples used to perform the platelet contractile force measurements.

It is important to note that both of these assays suffer from the lack of dynamic flow conditions and lack of in vivo agonist concentrations that truly reflect the conditions at the surgical wound. In addition, *Heparinase I* was added ex vivo to remove residual heparin prior to measuring the platelet contractile force assay given the sensitivity of this assay to the effects of heparin [19,30]. Collectively, these factors limit the ability to perfectly duplicate and detect conditions at the surgical wound—thus making this panel of assays potentially insensitive to the presence of an extrinsic defect (i.e., insufficient agonists) on platelet function in the patients we studied.

In summary, this study demonstrates that prolongation of CPB is related to increasing degrees of platelet dysfunction and that reductions in platelet contractile force measured in whole blood are related to decreases in platelet adhesion receptors and early postoperative blood loss. Given that platelet dysfunction is a major cause of blood loss following CPB, the use of the whole blood platelet contractile force measurement may be useful in providing a near-site method for monitoring of platelet function in patients undergoing cardiac surgery.

### Acknowledgements

The authors are indebted to all of the anesthesiology, surgery, nursing and research laboratory members of the Cardiothoracic Research Team.

The funding for this project was provided, in part, by the Department of Veterans Affairs (VISN #17 New Investigator Award) and the Society of Cardiovascular Anesthesiologists (Research Starter Grant).

### References

- [1] Harker LA, Malpass TW, Branson HE, Hessel EA, Slichter SJ. Mechanism of abnormal bleeding in patients undergoing cardiopulmonary bypass: acquired transient platelet dysfunction associated with selective  $\alpha$ -granule release. *Blood* 1980;56:824–34.
- [2] Kestin AS, Valeri CR, Khuri SF, Loscalzo J, Ellis PA, MacGregor H, Birjiniuk V, Ouimet H, Pasche B, Nelson MJ. The platelet function defect of cardiopulmonary bypass. *Blood* 1993;82:107–17.
- [3] Woodman RC, Harker LA. Bleeding complications associated with cardiopulmonary bypass. *Blood* 1990;76:1680–97.
- [4] Rinder CS, Mathew JP, Rinder HM, Bonan J, Ault KA, Smith BR. Modulation of platelet surface adhesion receptors during cardiopulmonary bypass. *Anesthesiology* 1991;75:563–70.
- [5] Ray MJ, Hawson GA, Just SJ, McLachlan G, O'Brien M. Relationship of platelet aggregation to bleeding after cardiopulmonary bypass. *Ann Thorac Surg* 1994;57:981–6.
- [6] Soslau G, Horrow J, Brodsky I. Effect of tranexamic acid on platelet ADP during extracorporeal circulation. *Am J Hematol* 1991;38:113–9.
- [7] George JN, Pickett EB, Saucerman S, McEver RP, Kunicki TJ, Kieffer N, Newman PJ. Platelet surface glycoproteins, studies resting and activated platelets and platelet membrane microparticles in normal subjects, and observations in patients during adult respiratory distress syndrome and cardiac surgery. *J Clin Invest* 1986;78:340–8.
- [8] Wenger RK, Lukasiewicz H, Mikuta BS, Niewiarowski S, Edmunds LH. Loss of platelet fibrinogen receptors during clinical cardiopulmonary bypass. *J Thorac Cardiovasc Surg* 1989;97:235–9.
- [9] van Oeveren W, Harder MP, Roozendaal KJ, Eijnsman L, Wildevuur CR. Aprotinin protects platelets against the initial effect of cardiopulmonary bypass. *J Thorac Cardiovasc Surg* 1990;99:788–97.
- [10] Maquelin KN, Berckmans RJ, Nieuwland R, Marianne CL, Have K, Eijnsman L, Sturk A. Disappearance of glycoprotein Ib from the platelet surface in pericardial blood during cardiopulmonary bypass. *J Thorac Cardiovasc Surg* 1998;115:1160–5.
- [11] Haan J, van Oeveren W. Platelets and soluble fibrin promote plasminogen activation causing downregulation of platelet glycoprotein Ib/IX complexes: protection by aprotinin. *Thromb Res* 1998;92:171–9.
- [12] Carr ME, Zekert SL. Measurement of platelet-mediated force development during plasma clot formation. *Am J Med Sci* 1991;302:13–8.
- [13] Carr ME, Carr SL, Hantgan RR, Braaten J. Glycoprotein IIb/IIIa blockade inhibits platelet-mediated force development and reduces gel elastic modulus. *Thromb Haemost* 1995;73:499–505.
- [14] Greilich PE, Carr ME, Carr SL, Chang AS. Reductions in platelet force development by cardiopulmonary bypass are associated with hemorrhage. *Anesth Analg* 1995;80:459–65.
- [15] Greilich PE, Alving BM, Longnecker D, Carr ME, Whitten CW, Chang AS, Reid TJ. Near-site monitoring of the antiplatelet drug abciximab using the Hemodyne analyzer and modified thrombelastograph. *J Cardiothorac Vasc Anesth* 1999;13:58–64.
- [16] Greilich PE, Alving BM, O'Neill KL, Chang AS, Reid TJ. A modified thromboelastographic method for monitoring c7E3 Fab in heparinized patients. *Anesth Analg* 1997;84:31–8.
- [17] Carr ME, Zekert SL. Force monitoring of clot retraction during DDAVP therapy for the qualitative platelet disorder of uraemia: report of a case. *Blood Coagul Fibrinolysis* 1991;2:303–8.
- [18] Friedenbergr WR, Myers WO, Plotka ED, Beathard JN, Kummer DJ, Gatlin PF, Stoiber DL, Ray JF, Sautter RD. Platelet dysfunction associated with cardiopulmonary bypass. *Ann Thorac Surg* 1978;25:298–305.
- [19] Carr ME, Carr SL, Greilich PE. Heparin ablates force development during platelet mediated clot retraction. *Thromb Haemostasis* 1996;75:674–8.
- [20] Ferraris VA, Ferraris SP, Singh A, Fuhr W, Koppel D, McKenna D, Rodriguez E, Reich H. The platelet thrombin receptor and postoperative bleeding. *Ann Thorac Surg* 1998;65:352–8.
- [21] Poullis M, Manning R, Laffan M, Haskard DO, Taylor KM, Landis RC. The antithrombotic effect of aprotinin: actions mediated via the protease activated receptor 1. *J Thorac Cardiovasc Surg* 2000;120:370–8.
- [22] Shattil SJ. Regulation of platelet anchorage and signaling by integrin  $\alpha_{IIb}\beta_3$ . *Thromb Haemostasis* 1993;70:224–8.
- [23] Carr ME, Carr SL. Fibrin structure and concentration alter clot elastic modulus but do not alter platelet mediated force development. *Blood Coagul Fibrinolysis* 1995;6:79–86.
- [24] Lee H, Nurden AT, Thomaidis A, Caen JP. Relationship between fibrinogen binding and the platelet glycoprotein deficiencies in Glanzmann's thrombasthenia type I and type II. *Br J Haematol* 1981;48:47–57.
- [25] Coller BS, Peerschke EI, Scudder LE, Sullivan CA. A murine monoclonal antibody that completely blocks the binding of fibrinogen to platelets produces a thrombasthenic-like state in normal platelets and binds to glycoproteins IIb and/or IIIa. *J Clin Invest* 1983;72:325–38.
- [26] Iijima K, Murata M, Nakamura K, Kitaguchi T, Handa M, Watanabe K, Fujimura Y, Yoshioka A, Ikeda Y. High shear stress attenuates agonist-induced, glycoprotein IIb/IIIa-mediated platelet aggregation when von Willebrand factor binding to glycoprotein Ib/IX is blocked. *Biochem Biophys Res Commun* 1997;233:796–800.

- [27] Adelman B, Michelson AD, Greenberg J, Handin RI. Proteolysis of platelet glycoprotein Ib by plasmin is facilitated by plasmin lysine-binding regions. *Blood* 1986;68:1280–4.
- [28] Kondo C, Tanaka K, Tkagi K, Shimono T, Shinpo H, Yada I. Platelet dysfunction during cardiopulmonary bypass surgery: with special reference to platelet membrane glycoproteins. *ASAIO J* 1993;39: M550–3.
- [29] Tabuchi N, de Haan J, Boonstra PW, Huet RC, van Oeveren W. Aprotinin effect on platelet function and clotting during cardiopulmonary. *Eur J Cardiothorac Surg* 1994;8:87–90.
- [30] Carr ME, Carr SL, Tildon T, Fisher LM. Comparison of thrombin- and batroxobin-induced clots reveals reduced platelet force in clotting factor deficient patients. *Blood* 1997;90:92b.