Original Article

Whole Blood Platelet Function Assay on the ICHOR™ Point-of-Care Hematology Analyzer

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ABSTRACT

The role of platelets as the initial defense against insult to the vasculature is well established. Moreover, platelets are now recognized as having a critical role in the acute care settings of cardiopulmonary bypass (CPB) procedures and cardiac catheterization. In the environment of CPB, both platelet count and function have been demonstrated as being markedly compromised during and following the procedure. Unfortunately, current assays that are used to evaluate the parameters of platelet count and function are limited in regard to their utility in a near patient format. Here, we describe a practical, rapid, and user-friendly whole blood platelet function assay that has been developed for the ICHOR™ point-of-care hematology analyzer. This analyzer is capable of performing an eight parameter blood profile including platelet count. In comparable studies, platelet aggregation in whole blood demonstrated good correlation (for ADP the values were n=14, r^2=0.81, p=0.0001; for collagen, n=10, r^2=0.93, p=0.0001; for ristocetin, n=10, r^2=0.89, p=0.0001; and for epinephrine, n=10, r^2=0.81, p=0.0003) with traditional platelet-rich aggregometry, which uses increased light transmission as an indication of platelet aggregation. Furthermore, early feasibility studies in CPB patients demonstrated both decreased platelet count and a marked reduction in platelet function peri-procedurally. This new assay of platelet function is extremely suitable for the clinical environment with rapid turnaround time and provides a full hematology profile to enhance transfusion decisions.

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INTRODUCTION

The anucleate, multifunctional platelet has a very important role in the first line of defense against any challenge to the hemostatic system. Subsequent to vascular injury, platelets adhere to collagen exposed as a result of endothelial denudation. At these sites, they change shape and release adenosine diphosphate (ADP), a physiological agonist which activates additional platelets. The primary hemostatic plug is achieved by the cyclic stimulation of platelets as they aggregate, release other agonists, and interact with the proteins of the coagulation cascade, forming a consolidated clot (1). However, if these processes are not closely regulated by homeostatic feedback mechanisms, the outcome may be either abnormal hemorrhage (bleeding) or thrombosis (clot formation) (2). In both of these pathological conditions, platelets play an important integral part.

Table 1: Some antiplatelet agents currently approved or in clinical trials

Antiplatelet Agent	Effect
Aspirin	Interferes with platelet thromboxane synthesis
ReoPro®	GPIIb/IIIa blockade (anticoagulant?)
Aggrastat®	GPIIb/IIIa blockade (synthetic)
Integrilin®	GPIIb/IIIa blockade (synthetic)
THA (Cognex®)	Multiple pathways—GPIIb/IIIa, reduce intracellular Ca++, other
Pentoxifylline	Increase in platelet cAMP and endothelial prostacyclin
Dipyridamole	Blocks erythrocyte adenosine uptake (phosphodiesterase inhibitor)
Dilazep	Blocks erythrocyte adenosine uptake
Triflusal	Inhibition of cyclooxygenase activity
Ticlopidine	Inhibits fibrinogen binding to platelet ADP receptors

The acute care environment of cardiac surgery, where cardiopulmonary bypass (CPB) is required, is one of several clinical settings where platelet function needs to be monitored. In cardiac surgery with CPB (of which more than 350,000 patients undergo procedures annually), many patients are at an increased risk of perioperative hemorrhage, with the subsequent requirement of transfusing blood products due to excessive activation and/or the consumption of platelets (3-5) or to underlying coagulopathies (6). Hemodilution during CPB may also affect the levels of the factors of the coagulation cascade predisposing the patient to excessive bleeding, which is further exacerbated by heparin and/or protamine dosing (7).

It is now well established that the activation and subsequent aggregation of platelets is the pivotal staging event for thrombus formation in acute coronary syndromes including myocardial infarction, unstable angina, and abrupt closure as a result of percutaneous transluminal coronary angioplasty (PTCA) (8-10). To permit optimal intervention in these clinical conditions it is now suggested that treatment regimens should utilize combination therapies of anticoagulation (AC), thrombolytics, and antiplatelet agents, all of which have demonstrated efficacy in significantly reducing the death rate associated with these syndromes (11-15). Indeed, in one study, a 35% reduction in the 30 day composite primary endpoint (death, non-fatal MI, or repeat vascularization) was achieved.

Although the benefits of proper AC and the use of thrombolytics have been recognized for some time, more recently, and following the demonstrated success of aspirin as an antiplatelet agent (16, 17), other, more effective, antiplatelet drugs have been developed. Some of these agents are outlined in Table 1. Although many of these agents have been tested in clinical trials, the first to become commercially available is the chimeric monoclonal antibody 7E3 Fab fragment known as abciximab[a] or ReoPro® (11-17). Targeting the platelet glycoprotein IIb/IIIa (GPIIb/IIIa) receptor, recognized as the penultimate step in platelet aggregation (18), this agent has been approved for use in high risk PTCA (11, 14, 17).

Clinical trials outlining the utility of this agent include the evaluation of c7E3 in preventing ischemic complications (EPIC trial). Here a 0.25 mg/kg bolus of ReoPro® was administered, and between 80 and 95% blockade of the platelet GPIIb/IIIa receptor was achieved. However, it was demonstrated that increased hemorrhage was associated with the regimen (19). The c7E3 antiplatelet therapy in unstable refractory angina (CAPTURE) study was designed to determine the utility of antiplatelet therapy in angioplasty patients diagnosed with unstable refractory angina (20). These patients were treated with ReoPro® for 18-24 hours pre-PTCA and for one hour peri-procedurally. Although a significant reduction in myocardial infarction, re-intervention, or death was observed at 30 days post-PTCA, no difference in overall cardiac event rates was demonstrated six months post procedure. It was therefore suggested that the short-term administration of ReoPro® may be inadequate to achieve maximal results (17, 20).

A major side effect associated with ReoPro® as an antiplatelet therapy has been an increased risk of hemorrhage (21). This has led to the suggestion that it is essential that the patient's hemostatic status is monitored sequentially throughout the procedure (22). As a non-specific antiplatelet agent, ReoPro® takes a "shotgun" approach to the inhibition of platelet aggregation, and as such it is of utmost importance to quantitate the level of platelet function to permit adequate dosing while minimizing hemorrhagic risk.

Traditional in vitro assessment of platelet function relies on the measurement of platelet aggregation in the presence of an agonist by optical methods (23, 25). Unfortunately, these methods are both laborious and indirect, since they utilize platelet-rich plasma (PRP) devoid of other elements of circulating blood (25), and as such these tests may not accurately reflect the in vivo condition where other blood components have a role in the

a Centocor and Eli Lily, Indianapolis, IN

modulation of platelet function. Recognized as having a greater physiological significance, whole blood platelet aggregation has been demonstrated using either increased impedance when platelets aggregate on electrodes, or by the inhibition of blood flow on a filter (25, 26). However, there are inherent limitations to both of these systems which include the natural tendency for platelets to become activated on a surface either in the presence or absence of an activator. Therefore, what is required for optimal determination of platelet function in acute care settings including CPB and PTCA is a near patient assay which accurately mimics the physiological state. Here we describe the utility of the ICHOR™ near patient hematology analyzer in the quantitation of platelet function and compare this mode of evaluation to the current laboratory standard: aggregation in PRP.

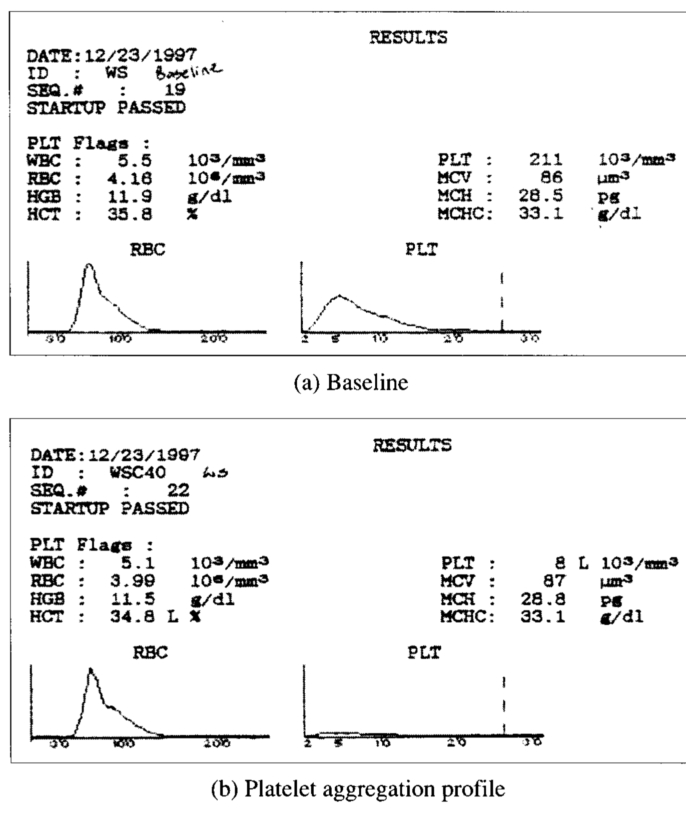


Figure 1: Representative printout demonstrating platelet aggregation (in presence of collagen) from the ICHOR

(a) Baseline

(b) Platelet aggregation profile

MATERIALS AND METHODS

THE ICHOR™ NEAR PATIENT HEMATOLOGY ANALYZER

Developed as a miniaturized near patient hematology analyzer, the ICHOR[b] currently provides an eight parameter complete blood count (CBC) measuring: the number of erythrocytes, leukocytes, platelets, direct quantitation of hematocrit, hemoglobin concentration, mean cell volume, mean corpuscular hemoglobin, and the mean corpuscular hemoglobin concentration. Using only 12 µL of whole blood, results for these parameters are provided in print-out format within two minutes. A typical readout is shown in Figure 1. Platelet function is measured on the ICHOR analyzer following initial CBC including baseline platelet measurement by a short (<5 min) incubation of whole blood in the presence of an agonist and quantitating the non-functional platelets in the sample.

REAGENTS

Vacutainers[c] containing 3.2% sodium citrate as the AC were used for sample collection for the processing of platelet-rich plasma. The ICHOR platelet function tubes were prepared using 3.2% sodium citrate and solutions of the following agonists: ristocetin, collagen, ADP, epinephrine, and thrombin[d]. These tubes were used to perform the aggregation assays on the ICHOR analyzer.

REAGENT STABILITY

To determine the stability of the reagents (anticoagulant mixed with agonist in solution) for platelet function evaluation, tubes were prepared with sodium citrate and the appropriate agonist—ADP, collagen, epinephrine, ristocetin, and thrombin at predetermined concentrations. These tubes were stored at -20°C. 2-8°C, 22-25°C (room temperature), and 37°C. The reagents were to be tested for stability at day = 0, 1, 2, 3, 4, 7, 14, weekly thereafter for one month, and monthly thereafter for one year as per NCCLS guidelines.

SAMPLES

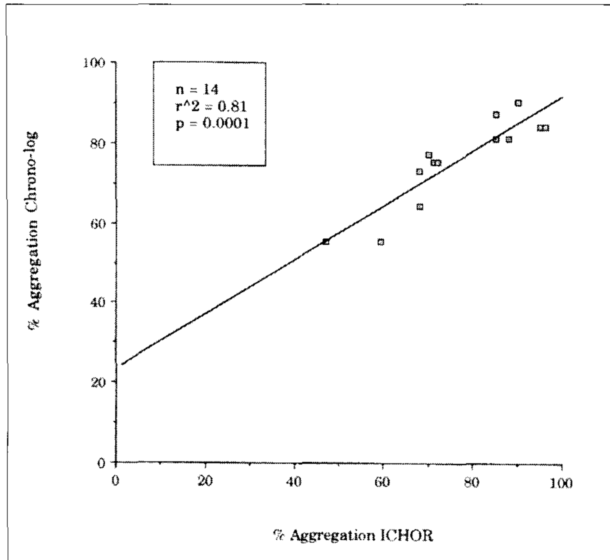
Following informed consent, blood samples were collected from healthy volunteers by careful venipuncture technique. Blood was collected without anticoagulant and was immediately aliquoted in 1 mL volumes into tubes containing 3.2% sodium citrate and the agonists described above. An additional tube containing whole blood and AC without agonist was used to establish baseline levels including a whole blood parameter determination as described.

PRP AGGREGATION

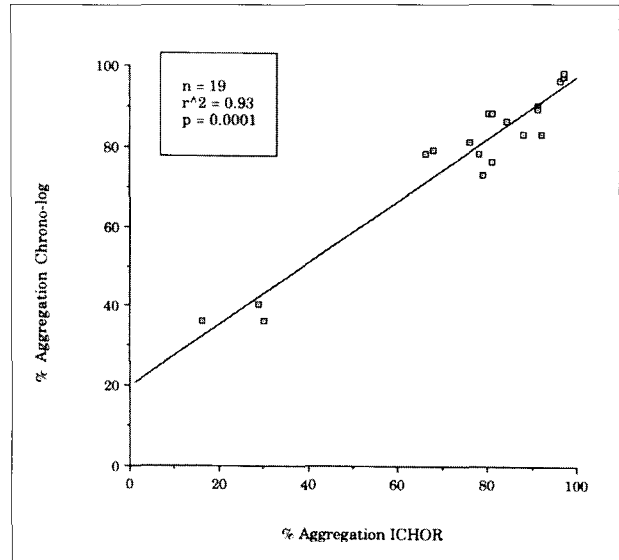
Samples from the same healthy voluteers were drawn into vacutainers containing 3.2% sodium citrate and were centrifuged at approximately 200 g for 10 minutes to provide PRP. The platelet depleted blood was re-centrifuged for 20 minutes at 2000 g to provide platelet poor plasma (PPP). PRP aggregation was measured using the Chronolog platelet aggregometer[e] against

b Array Medical, Somerville, NJ
c Becton Dickinson, Rutherford, NJ
d Sigma Diagnostics, St. Louis, MO
e Chrono-log, Haverton, PA

Figure 2: (a) Comparative ADP aggregation data (ICHOR vs. Chronolog); (b) Comparative collagen aggregation data (ICHOR vs. Chronolog); (c) Comparative epinephrine aggregation data (ICHOR vs. Chronolog); (d) Comparative ristocetin aggregation data (ICHOR vs. Chronolog)



(a)



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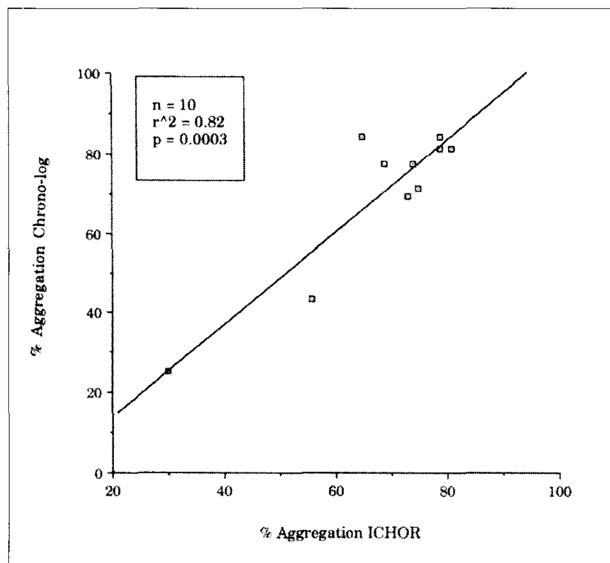
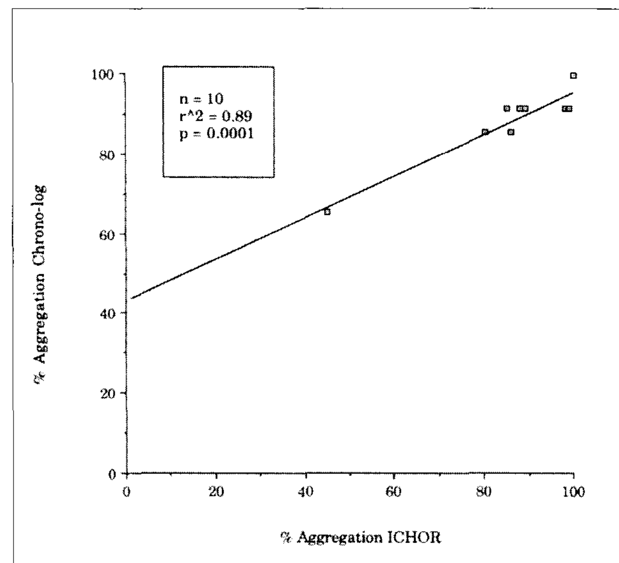


(c)



(d)

PPP. The testing was performed as per the manufacturer's guidelines.

CLINICAL STUDIES

During cardiac surgery where CPB is required, platelets play a very important role. A clinical feasibility study was conducted to test the utility of the ICHOR analyzer in this setting. Following approval by the Institutional Review Board (St. Joseph's Medical Center, South Bend, IN) and informed consent, blood samples from patients undergoing CPB were drawn into vacutainers containing buffered sodium citrate. Samples were collected pre, during, and post-procedurally. Within 60 minutes from collection, the blood was aliquoted in 1 mL volumes to tubes containing agonist. These samples were tested in the ICHOR analyzer. Again baseline levels were measured using whole blood samples in the absence of any agonist.

DESCRIPTION OF PLATELET FUNCTION ASSAY

The measurement of platelet function on the ICHOR analyzer uses tested cell counting hematology principles. The platelet count is performed in a diluted sample that is passed through an aperture, thereby interrupting a constant electrical current,

Table 2: Suggested aggregation levels for each agonist

Agonist	Suggested % Aggregation Achievable
ADP	83-100
Collagen	80-100
Epinephrine	67-97
Ristocetin	85-100
Thrombin*	90-100

*Not used in current studies

Figure 3: Representative platelet counts in CPB patients measured pre-CPB, during CPB, and at 2 and 24 hours post-CPB

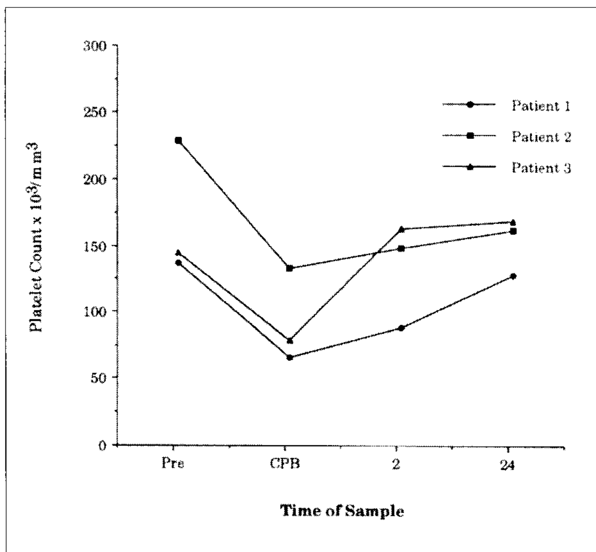
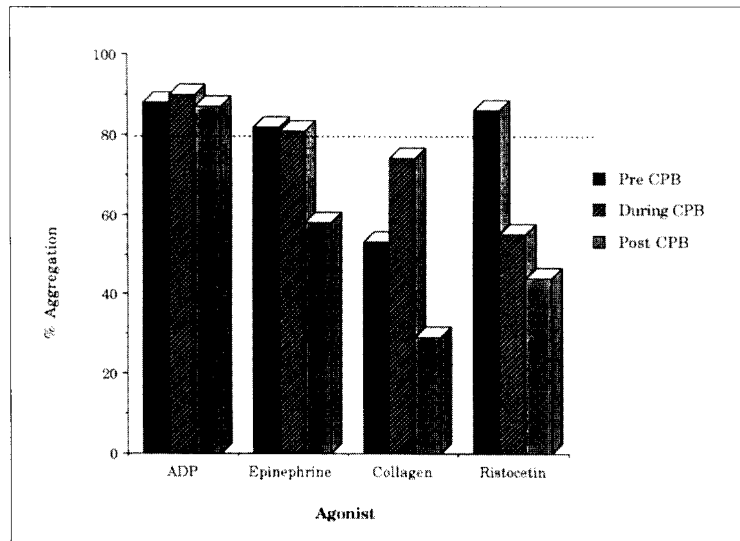


Figure 4: Platelet aggregation profile compared with baseline levels in a CPB patient in the presence of agonist



resulting in an electrical impulse which is amplified and sorted to provide the final platelet count in platelets X $10^3/mm^3$. In addition, the ICHOR analyzer is capable of providing a histogram of platelet size distribution (Figure 1). In the presence of an agonist, the platelets associate and aggregate. As aggregated platelets exceed the threshold limitations for platelet size, they are therefore no longer counted as a platelet. The differential between the tubes is representative of the functional platelets in the sample.

ANALYSIS

The platelet aggregation data ICHOR versus PRP aggregometry was calculated and is presented as graphs with correlation and probability values.

RESULTS

WHOLE BLOOD VS. PLATELET RICH PLASMA

Comparisons of platelet function in whole blood samples (n=53), taken from healthy volunteers, on the ICHOR analyzer with that of PRP on the Chronolog aggregometer are shown in Figure 2. In these tests, the analysis was performed on paired specimens. To assess the agonist concentration where standard aggregation was achieved as per literature values, all agonists were initially tested on samples from volunteers, who had not taken any substance known to affect platelet function for a minimum of seven days (Table 2). It was observed that WB platelet function demonstrated good correlation to the laboratory reference method using PRP with all agonists tested with the exception of thrombin (data not shown). For ADP, the values were $n = 14$, $r^2 = 0.81$, $p = 0.0001$; for collagen, $n = 19$, $r^2 = 0.93$, $p = 0.0001$; for ristocetin, $n = 10$, $r^2 = 0.89$, $p = 0.0001$; and for epinephrine, $n = 10$, $r^2 = 0.81$, $p = 0.0003$.

CPB PATIENTS PLATELET COUNT AND FUNCTION

In the feasibility study (n = 30) which was undertaken to determine the effect of CPB on both platelet count and function and the potential utility of the ICHOR in this acute care setting, blood samples were drawn into anticoagulant pre CPB, during the procedure, and in the post-procedural unit. Representative profiles of platelet counts for some of these patients are shown in Figure 3. It was demonstrated that in this study, CPB patients were observed to have a lower platelet count during the procedure, which supports the findings of other studies. In addition to reduced platelet count, it was observed that platelet function (comparing pre and post whole blood platelet aggregation in the presence of an agonist) in a number of patients was reduced post CPB. The aggregation profile of one such patient is shown in Figure 4. In this patient, normal platelet aggregation was observed in re-

sponse to ADP throughout the procedure, whereas marked reduction in platelet aggregation to epinephrine, collagen, and ristocetin was shown post CPB.

DISCUSSION

Although the critical role of platelets as the first line of defense against vessel injury has been recognized for some time, it is now realized that they are also crucial in the acute care settings of CPB and PTCA (10, 27). If dysfunctional (due to the nature of the procedure in CPB or the use of antiplatelet therapy in PTCA), serious hemorrhagic complications may occur. Indeed, one of the common side effects of antiplatelet therapy as demonstrated in clinical trials is bleeding (21). Furthermore, both reduced platelet count and excessive postoperative blood loss are common adverse events in CPB (27-29). It is therefore suggested that by monitoring platelet count and function in these clinical settings, enhanced platelet outcome may be achieved by minimizing the rate of morbidity and mortality and also by reducing the length of stay and the need for transfusing blood products. Unfortunately, traditional assays that provide quantitative evaluation of platelet count and function are either (a) laborious to perform; (b) not available on a near patient platform suitable for these settings; or (c) not directly representative of the physiological state (i.e., a PRP sample).

Here we describe the initial feasibility studies of platelet function on the ICHOR analyzer. This system has demonstrated utility in providing an eight parameter hematology profile and has recently been modified to perform platelet function evaluation. The method of determining platelet function is to measure total platelet count in a whole blood sample and then to redetermine the number of platelets in the sample in the presence of an agonist. The difference provides a direct measurement of platelet function and may be reported in percentage aggregation units.

In samples obtained from healthy volunteers, it was determined that platelet aggregation can be determined in whole blood very efficiently in under two minutes using the ICHOR system (Figure 1). Hence, the limitations of the traditional aggregation assays using optical measurements in platelet-rich plasma may be overcome (30-31). In comparative tests using samples from healthy volunteers, good correlation (r^2>0.8) was observed for all agonists tested (with the exception of thrombin), as shown in Figure 2. Although thrombin was tested, it was observed that, at suggested concentrations, rapid and total hemostasis (clotting) occurred in whole blood samples. It is suggested that this may be due to the interaction of this agonist with other blood components including fibrinogen. Additional studies are warranted to investigate platelet aggregability in whole blood in the presence of this agonist.

In the samples drawn from patients undergoing CPB procedures, it was demonstrated that there was a marked reduction in platelet count during the procedure that approximated normal values post procedure. This is in concordance with other studies (6). Prior to platelet transfusion, it is recommended (National Institutes of Health guidelines) that a platelet count <50,000/μL or that excessive or microvascular bleeding coupled with a platelet count <100,000μL be used as a reference (32). However, it has also been suggested that a normal platelet count (>150,000/μL) should not preclude platelet transfusion in conjunction with bleeding if platelet dysfunction is demonstrated. Currently, this is measured by the bleeding time assay, which is qualitative at best. These recommendations again support the immediate clinical need for a quantitative measurement of platelet count along with an accurate assessment of platelet function in a near patient format. These needs are met with the ICHOR system. Indeed, in the study described here, it was demonstrated that the ICHOR analyzer has potential utility to monitor platelet function in the acute care environment of CPB.

In conclusion, this feasibility study has demonstrated that both platelet count and function are easily measured in whole blood on the ICHOR analyzer. In addition to quantitation of platelet count and function, seven other hematologic parameters are provided which further facilitate patient risk stratification and should ultimately lead to improving the level of patient care. Furthermore, with the established relationship between inflammation and patient risk for adverse hemorrhagic events post CPB procedure (3), it has been demonstrated that the leukocyte count provides valuable diagnostic information predictive of bleeding risk. A leukocyte count is one of the baseline complete blood count parameters tested on the ICHOR. Additional large scale studies are warranted to determine the utility of this novel platform in the acute care settings of CPB and PTCA.

REFERENCES

1. Harker LA, Mann KG. Thrombosis and fibrinolysis. In: Fuster V, Verstraete M, eds. Thrombosis in cardiovascular disorders. Philadelphia: WB Saunders 1992:1-16.
2. Badimon L, Badimon JJ, Fuster V. Pathogenesis of thrombosis. In: Fuster V, Verstraete M, eds. Thrombosis in cardiovascular disorders. Philadelphia: WB Saunders 1992:17-39.
3. Despotis GJ, Levine VL, Goodnough LT. Relationship between leukocyte count and patient risk for excessive blood loss after cardiac surgery. Crit Care Med. 1997; 25: 1338-1346.
4. Sughayer M, Arkin CF. Monitoring coagulation during and after cardiopulmonary bypass surgery. Thromb Hemost. 1990; 12: 1-7.
5. Woodman RC, Harker LA. Bleeding complications associated with cardiopulmonary bypass: Review. Blood. 1990; 76: 1680-1697.
6. Khuri SF, Wolfe JA, Josa M, et al. Hematologic changes during and after cardiopulmonary bypass and their relationship to the bleeding time and nonsurgical blood loss. J Thorac Cardiovasc Surg. 1992; 104: 94-107.

7. Despotis GJ, Santoro SA, Spitznagel E, et al. Prospective evaluation and clinical utility of on-site monitoring of patients undergoing cardiac operation. J Thorac Cardiovasc Surg. 1994; 107: 271-279.
8. De Wood MA, Spores J, Notscke R, et al. Prevalence of total coronary occlusion during the early hours of transmural myocardial infarction. N Engl J Med. 1980; 303: 897-905.
9. Ammar T, Scudder LE, Coller BS. In vitro effects of the platelet glycoprotein IIb/IIIa receptor antagonist c7E3 Fab on the activated clotting time. Circulation. 1997; 95: 614-617.
10. Califf RA, Willerson JT. Percutaneous transluminal coronary angioplasty: prevention of occlusion and restenosis. In: Fuster V, Verstraete M, eds. Thrombosis in cardiovascular disorders. Philadelphia: WB Saunders 1992; 389-408.
11. Anderson HV, Jordan RE, Weisman HF. Concept and clinical application of platelets glycoprotein IIb/IIIa inhibition with abciximab (c7E3 Fab; ReoPro) for the prevention of acute ischemic syndromes.
12. Antiplatelet Trialists Collaboration. Collaborative overview of randomized trials of antiplatelet therapy. I: Prevention of death, myocardial infarction, and stroke by prolonged antiplatelet therapy in various categories of patients. Br Med J. 1994; 308: 81-91.
13. Coller BS. A new murine monoclonal antibody reports on activation-dependent change in the conformation and/or microenvironment of the platelet glycoprotein IIb/IIIa complex. J Clin Invest. 1985; 76: 101-108.
14. Moliterno DJ, Califf RM, Aguirre FV, et al. Effect of platelet glycoprotein IIb/IIIa integrin blockade on activated clotting time during percutaneous transluminal coronary angioplasty or directional atherectomy (the EPIC) trial. Am J Cardiol. 1995; 75: 559-562.
15. Moditerno DJ, Topol EJ. Conjunctive use of platelet glycoprotein IIb/IIIa antagonist and thrombolytic therapy for acute myocardial information. Thromb Haem. 1997; 78: 214-219.
16. Schwartz L, Bourassa MG, Lesperance L, et al. Aspirin and dypridamole in the prevention of restenosis after percutaneous transluminal coronary angioplasty. N Engl J Med. 1988; 318: 1714-1719.
17. Tcheng JE. Platelet glycoprotein IIb/IIIa integrin blockade: recent clinical trial in interventional cardiology. Thromb Haem. 1997; 78: 205-209.
18. Plow EF, Ginsberg MH. Cellular adhesion: GPIIb/IIIa as a prototypic adhesion receptor. Prog Hemost Thromb. 1988; 9: 117-156.
19. Tcheng JE, Ellis SG, George BS, et al. Pharmacodynamics of chimeric glycoprotein IIb/IIa integrin antiplatelet antibody Fab 7E3 in high risk coronary angioplasty. Circulation 1994; 90: 1757-1764.
20. Simoons ML, de Boer MJ, van der Brand MJBM, et al. Randomized trial of a GPIIb/IIIa platelet receptor blocker in refractory unstable angina. Circulation 1994; 89: 596-603.
21. Lincoff AM, Tcheng JE, Califf RM, et al. Standard versus low-dose weight adjusted heparin in patients treated with the platelet glycoprotein IIb/IIIa receptor antibody fragment abciximab (c7E3 Fab) during percutaneous coronary revascularization. Am J Cardiol. 1997; 79: 286-291.
22. Ogilby JD, Carville DGM. Anticoagulant management in interventional cardiology (monitoring heparin using the activated clotting time instrumentation). Cardio Rev Reports. 1998; November: 34-47.
23. Born GVR, Cross MJ. The aggregation of blood platelets. J Physiol. 1963; 168: 178-195.
24. Born GVR, Hardisty RM. Platelets. In: Biggs R, ed. Human blood coagulation haemostasis and thrombosis, 2nd Ed. London: Blackwell Scientific Publications. 1976; 168-201.
25. Dawicki DD, Argawal KC, Parks RE. Role of adenosine uptake and metabolism by blood cells in the antiplatelet actions of dipyridamole, dilazep, and nitrobenzylthioinosine. Biochem Pharmacol. 1985; 34: 3965-3972.
26. Dade PFA-100™. Platelet function analyzer. Technical information. 1997.
27. Ray MJ, Hawson GAT, Just SJE, McLachlan G. O'Brien M. Relationship of platelet aggregation to bleeding after cardiopulmonary bypass. Ann Thorac Surg. 1994; 57: 981-986.
28. Bachmann F, McKenna R, Cole ER, Najafi H. The hemostatic mechanism after open heart surgery. I: Studies on plasma coagulation factors and fibrinolysis in 512 patients after extracorporeal circulation. J Thorac Cardiovasc Surg. 1975; 70: 76-85.
29. Campbell FW, Addonzio VP. Platelet function alterations during cardiac surgery. In: Ellison N, Jobes DR, eds. Effective hemostasis in cardiac surgery, 1st Ed. Philadelphia: WB Saunders, 1988: 85-109.
30. Riess H, Braun G, Brehm G, Hiller E. Critical evaluation of platelet aggregation in whole human blood.
31. Podczasy JJ, Lee J, Vucenik I. Evaluation of whole-blood lumiaggregation. Clin Appl Thromb/Hemost. 1997; 3: 190-195.
32. National Institutes of Health Consensus Conference. Platelet transfusion therapy. JAMA. 1987; 257: 1777-1780.