

Original Article

Clinical Comparison of Patient-Side Fibrinogen Assay and Common Laboratory Analyzer in Pediatric Cardiopulmonary Bypass

Dorothy R. Matthews*; Jodie M. Ecklund, BS, CCP*; Hani Hennein, MD**

*Program of Extracorporeal Circulation Technology, College of Health Professions and **Department of Cardiothoracic Surgery, Medical University of South Carolina, Charleston, South Carolina

Presented at the 33rd International AmSECT Meeting, Orlando, Florida, April 26-30, 1995

Keywords: fibrinogen, hemostasis, coagulation, patient-side assay

ABSTRACT

The coagulation status of infant and pediatric patients can be severely compromised during the course of cardiopulmonary bypass due primarily to hemodilution and hypothermia. Fibrinogen level is one source of information necessary to assess the coagulation status of a patient. An accurate and expedient method to determine the fibrinogen level would allow for earlier initiation of coagulation therapy to prevent excessive postoperative bleeding. The purpose of this study was to compare two methods of determining fibrinogen level: a patient-side assay and a common laboratory analyzer. The patient-side test utilized the HemoChron Fibrinogen Assay and was performed in the operating room. The MLA 1000C was the laboratory method utilized in the hospital's coagulation laboratory. Simultaneous testing was conducted prebypass and intraoperatively on 26 infant and pediatric patients undergoing cardiopulmonary bypass for palliation and correction of congenital heart defects. The resulting values were compared using paired t-test, regression and correlation analysis, and descriptive analysis. The values obtained by the two methods were significantly different ($p < .05$) at each collection time. Further analysis revealed that other variables, such as hematocrit and platelet count, affected the differences between the results of the methods. The HemoChron Fibrinogen Assay may not be a viable tool for the assessment of fibrinogen level on infant and pediatric patients undergoing cardiopulmonary bypass surgery. Further studies should be done in this patient population incorporating other confounding variables.

Address correspondence to:
Dorothy R. Matthews
Medical University of South Carolina
101 Doughty Street, 2nd Floor
Charleston, SC 29401

INTRODUCTION

Hemostatic abnormalities are inevitably associated with cardiopulmonary bypass (CPB). There are many contributing factors to hemostatic abnormalities which include exposure to foreign surfaces, hyperfibrinolysis, reduced platelet function, incomplete heparin reversal, hypothermia, and disseminated intravascular coagulation (1). Reduction in clotting factors due to hemodilution can be significant in infant and pediatric patients because the priming volume for the extracorporeal circuit (ECC) is greater than their blood volume (2). Hypothermia contributes to the dysfunction by retarding the enzymatic activation of coagulation factors (3). Additionally, children with cyanotic congenital heart disease tend to have a pre-existing coagulation dysfunction (4). Along with maintaining heparinization (ACT>480sec), there is an increased potential for excessive bleeding during and after surgery.

Postoperatively the focus is on returning to a normal or near-normal coagulation state. This is achieved by obtaining and maintaining a balance between coagulation and fibrinolysis to prevent excessive hemorrhage and thrombosis (5). Determining when such a balance has been obtained is assisted by various coagulation tests, each focusing on a different aspect of coagulation. These tests are instrumental in evaluating not only when hemostatic balance has been achieved, but also in providing information as to the cause of continuing postoperative bleeding (6). Some of the commonly used tests are prothrombin time (PT), activated partial thromboplastin time (aPTT), activated clotting time (ACT), thrombin time (TT), platelet count (PLT) and fibrinogen level.

The PT assesses the extrinsic and common limbs of the coagulation cascade and a prolonged PT may indicate coumadin therapy, vitamin K deficiency, Factor VII deficiency, or high doses of heparin. The aPTT assesses the intrinsic and common limbs of the coagulation cascade. If prolonged, it could indicate deficiencies of Factors VII, XII, IX, XI, or low doses of heparin (7). The TT assesses the conversion of fibrinogen to fibrin. A fibrinogen level provides information as to ability to form and stabilize a fibrin clot (8). The presence of heparin and decreased fibrinogen can prolong the TT (9).

While these tests offer valuable information, the time in which the information is made available to health care workers is suboptimal (10). Much of the time is spent in transporting the sample and preparing the sample for processing (11). Near-patient or patient-side testing is a means of providing results quicker than traditional laboratory methods by eliminating the need for transport and timely sample preparation because whole blood is utilized for testing. It produces a rapid turnaround time and conserves blood volume because a much smaller sample volume is needed to perform the test (12).

There is an increasing availability of patient-side testing, especially in intensive care units, emergency areas, satellite labs, and operating rooms (13). Most laboratories are equipped to run various coagulation tests, one of which is a fibrinogen level. A

fibrinogen level is routinely requested after cardiac surgery on infant and pediatric patients to assess their clotting ability and determine the necessity of fresh frozen plasma or cryoprecipitate administration. Because the coagulation status of these patients has been further compromised by the effects of surgery, a rapid assessment of their coagulation status allows for the initiation of prompt corrective therapy. Currently, it can take up fifteen minutes or more to receive the results from the laboratory (14). An alternative to the delay is a patient side test that can be done in the operating room in less time.

Although it is advantageous to receive results rapidly, it is more important to have consistently accurate and reliable results. Thus, the focus of this study is to compare the fibrinogen level obtained using a patient-side assay to accepted laboratory results. The null hypothesis states that there is no significant difference in the fibrinogen level obtained using the patient-side assay versus a traditional laboratory method. If the null hypothesis is not rejected, the patient-side test may be utilized in the operating room as an indicator of fibrinogen concentration.

MATERIALS & METHODS

The study group consisted of twenty six infant and pediatric patients undergoing open heart surgery and CPB for various palliative or corrective procedures. Patient selection was based primarily on weight (less than 45 kg) exclusive of surgeon or procedure. Fibrinogen level was determined prebypass and intraoperatively during the rewarming phase of CPB. The prebypass sample was collected by anesthesia via the central line and the intraoperative sample was collected by the perfusionist via the arterial sampling port on the manifold of the ECC. Each sample contained six milliliters (ml) of blood which was shared for simultaneous testing.

Five ml was transferred to a blue-top citrated specimen tube^a and transported to the coagulation lab for analysis. The coagulation lab utilized the MLA 1000C^b which required a minimum of 4.7 ml of whole blood to insure 2 ml of serum could be obtained after the sample was rotated at a speed of 2000 rpm for 4 minutes in a centrifuge. The MLA 1000C is a photo-optic test that detects changes in the opaqueness of the sample. Thrombin is the active reagent. The HemoChron Fibrinogen Assay^c (HFA) test tube contains 20 mg of lyophilized preparation of human thrombin, snake venom, extract form B, atrox, protamine sulfate, buffers, calcium stabilizer and thimersol. A clotting time is obtained utilizing the HemoChron 8000 coagulation analyzer^c which detects clot formation by displacement of a magnet within the test tube. Based on the clotting time, a fibrinogen level was internally extrapolated from a standardized graph developed by International Technidyne Corporation (ITC). Sample size for the HFA

a Baxter Diagnostic Inc., Deerfield, IL 60001-4633

b Medical Laboratory Analyzer, Pleasantville, NY 10572

c International Technidyne Corporation, Edison, NJ 08820

test was dependent upon whether or not the test performed would be diluted or non-diluted. This determination was based upon an expected fibrinogen value of 120 mg/dl. For values less than 120 mg/dl, a non diluted test was performed. A diluted test was performed on values greater than 120 mg/dl per manufacturer recommendations. In a pilot-test of five patients, three of the samples failed to clot, resulting in a "no conversion" result from the HFA (i.e. no fibrinogen value could be computed). A diluted and undiluted test were simultaneously done on the other two samples. Because the results showed that the diluted samples were lower than the undiluted samples on the same blood sample, the remaining tests for the study were not diluted. To assess equipment function, abnormal and normal plasma controls were performed prior to each test. Other variables recorded included: PLT, hematocrit (HCT), and activated clotting time (ACT). The data was statistically analyzed using descriptive tests, paired t-test, ANOVA, regression, correlation and residual analysis. A p value <0.05 was chosen to assess statistical significance.

RESULTS

Tables 1 and 2 show the descriptive data for the two methods. The mean prebypass fibrinogen for the laboratory and HFA were 199.8 mg/dl and 149.8 mg/dl, respectively. The intraoperative means were 110.7 mg/dl for the lab and 92.3 mg/dl for the HFA. A paired t-test was performed to evaluate the significance of the difference between the two means. The test revealed significant differences with a prebypass mean difference of 50 mg/dl and intraoperative mean difference of 18.41 mg/dl (Table 3). Regression and correlation analyses were performed to determine the relationship between the fibrinogen values. There was no significant correlation between the fibrinogen level obtain by the two methods (prebypass $r=.303$, intraoperative $r=.23$, $p=NS$). Figures 1 and 2 are scatterplots of the fibrinogen values for each method for the two sampling periods with their respective regression equations.

Residual plots of the regression of laboratory's fibrinogen level on the HFA's level revealed the necessity to look at other variables for inclusion in the multiple linear regression analysis. Further analysis including ACT, HCT and PLT revealed that the HCT and PLT significantly affected the difference between the intraoperative fibrinogen values ($r=.85$, $p=0.001$). Partial t-

Table 1: Descriptive statistics for the fibrinogen values obtained pre-bypass for the two methods. Lab=laboratory analyzer, HFA=HemoChron fibrinogen assay

	Mean	Standard Deviation
HFA	149.8 mg/dl	51.3
Lab	199.8 mg/dl	32.3

n=25

Table 2: Descriptive statistics for the fibrinogen values obtained intra-operatively for the two methods. Lab=laboratory analyzer, HFA=HemoChron fibrinogen assay

	Mean	Standard Deviation
HFA	92.3 mg/dl	25.8
Lab	110.7 mg/dl	24.3

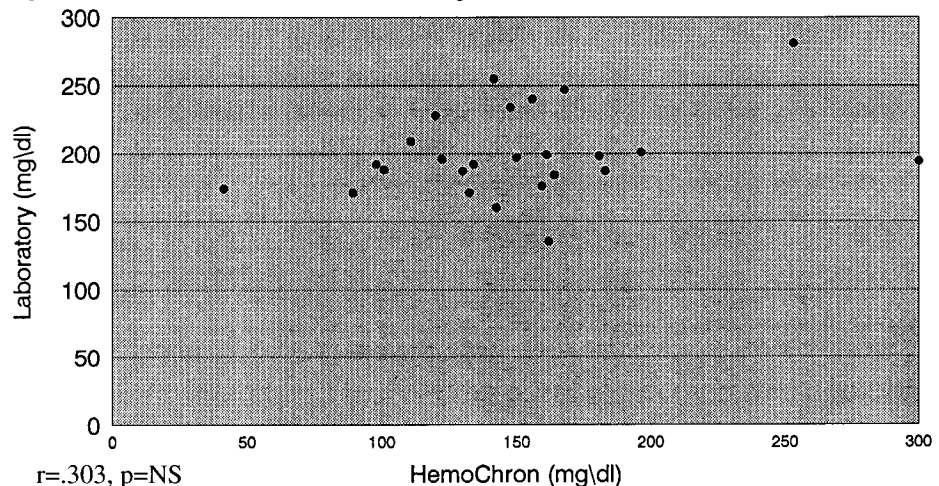
n=26

Table 3: Results of the paired t-test analysis comparing the two methods. Lab=laboratory analyzer, HFA=HemoChron fibrinogen assay

	Mean Difference	Standard Deviation	p-value
Pre-bypass	-50.00 mg/dl	51.70	0.0001
Intra-operative	-18.41 mg/dl	31.14	0.0058

Mean difference = HFA-lab

Figure 1: Scatterplot of the intra-operative fibrinogen values obtained by the two methods. r =Spearman's correlation coefficient, NS=not significant



test results and the regression equation are shown in Table 4. Figures 3 and 4 are graphical representations of these relationships. In Figure 3, as the PLT decreases, the difference also decreases. Similarly in Figure 4, as the HCT decreases, the difference decreases.

DISCUSSION

Coagulation tests are vital for the proper assessment and treatment of infant and pediatric patients undergoing CPB. An accurate patient-side test for fibrinogen level would allow for rapid assessment of clot stability and prompt initiation of corrective therapy. Analysis of the data collected suggested that the fibrinogen value obtained from the HFA was not comparable to the laboratory value. The HFA demonstrated consistently lower fibrinogen levels than the laboratory at both collection times.

Variables such as ACT, HCT and PLT could have attributed to the discrepancy between the results. These variables are more likely to affect the HFA because it is a whole blood test, whereas the laboratory test is performed only on plasma. Statistical analysis of the intraoperative values reveal a strong correlation ($r=.85$) between PLT, HCT, and the fibrinogen difference. Figure 4 shows a greater difference at higher hematocrit values. Similarly, at higher platelet counts, there is increased difference between the fibrinogen values (Figure 3). None of these variables was found to affect the difference in prebypass fibrinogen values. Platelet counts used in the prebypass data were obtained from samples drawn the evening prior to surgery, whereas the hematocrit and fibrinogen were redrawn prior to CPB. Analysis of the change in fibrinogen and hematocrit from the evening before surgery revealed that dilution by anesthesia significantly reduced these values. It is expected that platelet counts would have revealed a similar trend. If the platelet count had been drawn prior to CPB, the linear regression results may have differed.

High heparin concentrations could have also contributed to the difference in the intraoperative fibrinogen results. The HFA's maximum reported accuracy is at a heparin concentration of 3.0 u/ml. Increasing heparin concentrations result in decreased fibrinogen values (Horrow, et al. "Fibrinogen Measurement for Hemostasis Assessment During Cardiac Surgery". Presented in XIVth Congress of the International Society on Thrombosis and Hemostasis). The laboratory test is less affected by high heparin concentrations. The goal at this institution is to obtain an initial

Figure 2: Scatterplot of the intra-operative fibrinogen values obtained by the two methods.
 r =Spearman's coefficient, NS=not significant

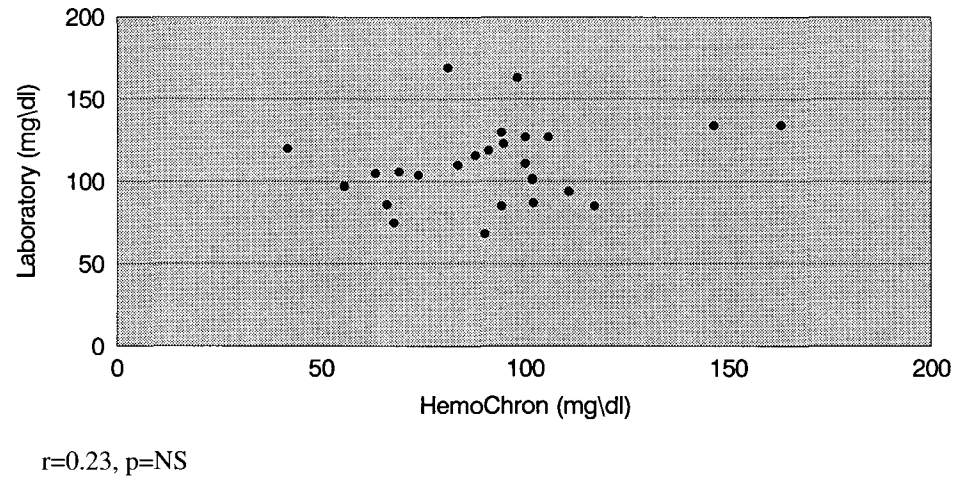


Table 4: Results of the multiple linear regression analysis on the fibrinogen difference between the two methods. HCT=hematocrit, PLT=platelet count, FIB Diff=HFA-lab, Lab=laboratory analyzer, HFA=HemoChron fibrinogen assay

Predictor	Coefficient	Standard Deviation	p-value
Hematocrit	-2.9613	0.7725	0.002
Platelets	-0.8518	0.2003	0.001

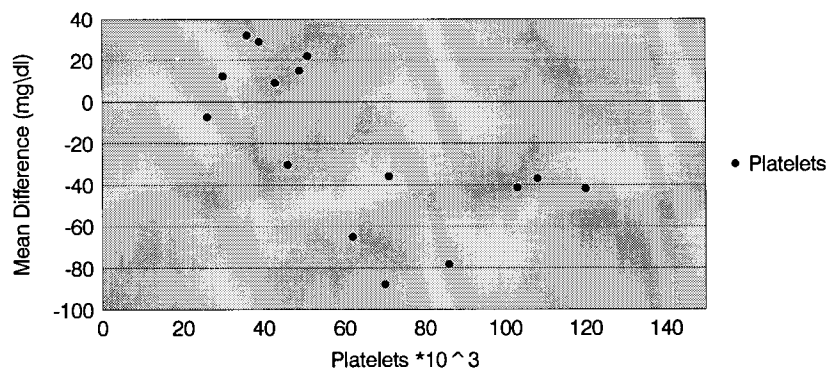
$r=0.85$, $p<0.05$

heparin concentration of 3.5 u/ml and then maintain an ACT of 480 seconds during surgery, which may help explain the observed differences.

Another contributing factor could be operator error and/or protocol variation. A total of 35 patients participated in the study; however, nine of the intraoperative and ten of the pre bypass samples did not clot in the HFA tube. There are multiple steps in performing the HFA in which an error may occur that could affect the result. For example, more (or less) than one ml of diluent in the tube would produce erroneous results. Nine individuals were responsible for performing the HFA for this study. Although everyone was trained to perform the HFA prior to data collection, the competency level of each individual varied with the number of times they actually performed the test.

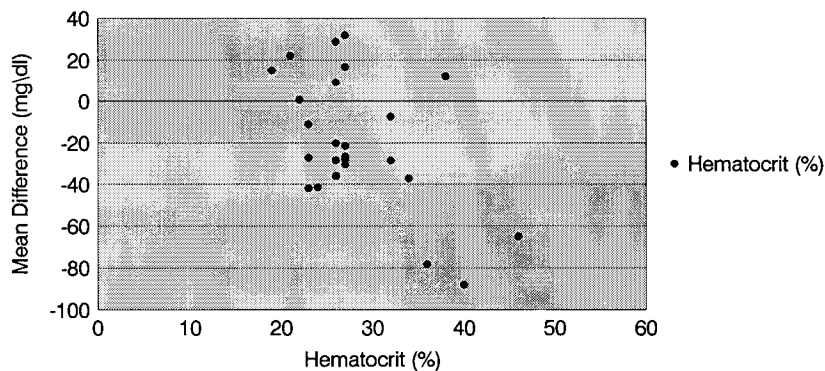
The standard curve from which the HFA fibrinogen level is extrapolated was derived from studies of adult patients with an average hematocrit of approximately 35% (range 20-50%). The hematocrits in this study were within this range, but the patient populations differed. The effect of platelet count on the HFA results was not considered in the development of the standard

Figure 3: Scatterplot of the difference between the intra-operative fibrinogen values obtained by the two methods versus the intra-operative platelet count. HFA=HemoChron fibrinogen assay.



Mean difference = HFA - Laboratory

Figure 4: Scatterplot of the difference between the intra-operative fibrinogen values obtained by the two methods versus the intra-operative hematocrit. HFA=HemoChron fibrinogen assay



Mean difference = HFA - Laboratory

curve (personal communication: Dr. Pan 14 Nov 95). This may be an important consideration for infant and pediatric patients, because their clotting factors and platelet counts are reduced more than adult patients' secondary to hemodilution. Perhaps the curve HFA uses to derive fibrinogen level is not applicable to infant and pediatric patients. Further studies should be done to evaluate the need for a standard curve for this patient population incorporating other confounding variables.

CONCLUSION

In conclusion we reject the null hypothesis that there is no difference between the fibrinogen level obtained from the HFA and the lab. Because statistical analysis revealed the differences

to be significant and affected by other variables, the HFA may not a viable tool to evaluate the fibrinogen level of infant and pediatric patients undergoing cardiopulmonary bypass surgery.

ACKNOWLEDGEMENTS

The authors would like to thank the ECT faculty and graduating class of 1995 for their assistance in data collection for this study and Agnes Hoff in the ITC Research Department for providing the supplies used in this study.

REFERENCES

1. Gravlee GP, Arora S, Lavender SW, et al. Predictive value of blood clotting tests in cardiac surgical patients. *Ann Thorac Surg.* 1194;58:216-21.
2. LeBlanc JG. Postoperative complications. In: LeBlanc JG, Williams WG, eds. *The operative and postoperative management of congenital heart disease.* New York: Futura; 1193;245.
3. Harrow JC. Management of coagulopathy associated with cardiopulmonary bypass surgery. In: Gravlee G, Davis R, Utley J, eds. *Cardiopulmonary Bypass: Principles and Practices.* Baltimore: Williams and Wilkins; 1993;450-451.
4. LeBlanc JG. Postoperative complications. In: LeBlanc JG, Williams WG, eds. *The Operative and Postoperative Management of Congenital Heart Disease.* New York: Futura; 1193;243.
5. Shafer KE, Santoro SA, Sobel BE, Jaffe AS. Monitoring activity of fibrinolytic agents. *Am J Med.* 1984;76:879-885.
6. Teufelsbauer H, Proidl S, Havel M, Vukovich TH. Early activation of hemostasis during cardiopulmonary bypass: Evidence for thrombin mediated hyperfibrinolysis. *Thrombosis and Haemostasis.* 1992;68(3):250-252.
7. Reich DL, Yanakakis MJ, Vela Cantos FP, DePerio M, Jacobs E. Comparison of bedside coagulation monitoring test with standard laboratory test in patients after cardiac surgery. *Anesth Analg.* 1993;77:673-9.
8. Gaffney PJ, Wong MY. Collaborative study of a proposed international standard for plasma fibrinogen measurement. *Thrombosis and Haemostasis.* 1992;68(4):428-32.
9. Shafer KE, Santoro SA, Sobel BE, Jaffe AS. Monitoring

- activity of fibrinolytic agents. *Am J Med.* 1984;76:879-885.
10. Farr AD. Near patient 'laboratory' testing. *Med Lab Sci.* 1990;47:249-250.
 11. Salem M, Chernow B, Burke R, Stacey J, Slogoff M, Sood S. Bedside diagnostic blood testing. *JAMA.* 1991; 266(3):382- 89.
 12. Kost GJ, Shirey TL. New whole blood testing for laboratory support of critical care at cardiac transplant centers and US hospitals. *Arch Pathol Lab Med.* 1990;114:868.
 13. Kost GJ. New whole blood analyzers and their impact on cardiac and critical care. *Crit Rev Clin Lab Sci.* 1993;30(2);153-202.
 14. Nelson S, Pritt A, Marlar RA. Rapid preparation of plasma for 'stat' coagulation testing. *Arch Pathol Lab Med.* 1994;118:175-76.