# Profound Effects of Cardiopulmonary Bypass Priming Solutions on the Fibrin Part of Clot Formation: An Ex Vivo Evaluation Using Rotation Thromboelastometry

Arinda C.M. Brinkman, MD,* Johannes W.A. Romijn, MD,* Lerau J.M. van Barneveld, MSc,† Sjoerd Greuters, MD,* Dennis Veerhoek, MSc,† Alexander B.A. Vonk, MD,† and Christa Boer, PhD*

***Objectives:*** **Dilutional coagulopathy as a consequence of cardiopulmonary bypass (CPB) system priming may also be affected by the composition of the priming solution. The direct effects of distinct priming solutions on fibrinogen, one of the foremost limiting factors during dilutional coagulopathy, have been minimally evaluated. Therefore, the authors investigated whether hemodilution with different priming solutions distinctly affects the fibrinogen-mediated step in whole blood clot formation.**

***Design:*** **Prospective observational laboratory study.**

***Setting:*** **University hospital laboratory.**

***Participants:*** **Eight male healthy volunteers.**

***Interventions:*** **Blood samples diluted with gelatin-, albumin-, or hydroxyethyl starch (HES)-based priming solutions were ex-vivo evaluated for clot formation by rotational thromboelastometry.**

***Measurements and Main Results:*** **The intrinsic pathway (INTEM) coagulation time increased from 186 ± 19 seconds to 205 ± 16, 220 ± 17, and 223 ± 18 seconds after dilution with gelatin-, albumin-, or HES-containing prime solutions (all $p < 0.05$ *v* baseline). The extrinsic pathway (EXTEM) coagulation time was only minimally affected by hemodilution. Moreover, all 3 priming solutions significantly reduced the INTEM and EXTEM maximum clot firmness. The HES-containing priming solution induced the largest decrease in the maximum clot firmness attributed to fibrinogen, from 13 ± 1 mm (baseline) to 6 ± 1 mm ($p < 0.01$ *v* baseline).**

***Conclusions:*** **All studied priming solutions prolonged coagulation time and decreased clot formation, but the fibrinogen-limiting effect was the most profound for the HES-containing priming solution. These results suggest that the composition of priming solutions may distinctly affect blood clot formation, in particular with respect to the fibrinogen component in hemostasis.**



***KEYWORDS: cardiothoracic surgery, hemostasis, hemodilution, coagulopathy, thromboelastometry, fibrinogen, HES, albumin, gelatin***

Dilutional coagulopathy during cardiopulmonary bypass (CPB) is of major concern in patients undergoing cardiac surgery. Hemodilution is not only associated with a decrease in hematocrit, but also is related to decreased levels of platelets, fibrinogen, and distinct clotting factors like antithrombin and plasminogen.[1-3] Moreover, extracorporeal circuit priming solutions contain a variety of components that may alter hemostasis during and after CPB. In the last decades, many investigations focused on the relation between the composition of priming solutions and postoperative bleeding. For example, several research groups studied the effect of replacement of the expensive component albumin by colloidal solutions containing hydroxyethyl starch (HES), hetastarch, or gelatin in extracorporeal bypass priming solutions on patient hemostasis. It was shown that, in addition to a significant cost reduction, these starches were not associated with more bleeding after surgery and, therefore, they were safe replacements for albumin during CPB.[4-7] To date, the number of distinct priming solutions used by cardiosurgical institutions worldwide is numerous. Lilley[8] surveyed 31 cardiac surgical centers in the United Kingdom and Ireland and found that no 2 units used similar compositions of priming.

Natural colloids like albumin are expensive, but they are associated with a lower risk of anaphylactic shock than cheaper artificial colloids like gelatin solutions.[9,10] Moreover, volume expansion with gelatin and albumin induces fewer coagulation abnormalities than HES.[11-14] Interestingly, these effects were most profound for fibrinogen, showing that colloid administration may critically impair fibrinogen polymerization and reduce fibrinogen concentrations.[15-17]

Point-of-care rotation thromboelastometry, a viscoelastic method for whole-blood hemostasis testing, is used in an increasing number of European countries for bedside coagulation monitoring in patients undergoing cardiothoracic surgery. This technique allows specific evaluation of the contact phase of hemostasis (INTEM) in the presence or absence of heparinase, screening of the extrinsic hemostasis system (EXTEM), and analysis of the fibrin part of the clot (FIBTEM).

The direct effect of CPB priming solutions on fibrinogen has been minimally investigated. In the present study, the authors hypothesized that clot formation as measured by thromboelastometry, in particular the fibrin part of the clot, is distinctly altered by different priming solutions. Using an ex vivo experimental setup, CPB priming solutions containing gelatin, albumin, or HES were compared with respect to their impact on coagulation time, clot firmness, fibrinogen, and the shear elastic modus. The present study may contribute to insight into mechanisms that may be responsible for hemostatic disturbances during CPB.

## METHODS

Thromboelastometric values were determined in blood samples of 8 healthy male volunteers. Data were obtained in accordance with the Local Human Subjects Division guidelines, and volunteers gave informed consent. None of the volunteers suffered from cardiopulmonary or hemostatic disorders or used medication that may affect hemostasis. Blood (4.5 mL) was drawn from all volunteers using

*From the Departments of *Anesthesiology and †Cardiothoracic Surgery, Institute for Cardiovascular Research, VU University Medical Center, Amsterdam, The Netherlands.*

*Address reprint requests to Christa Boer, PhD, Department of Anesthesiology, VU University Medical Center, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands. E-mail: c.boer@vumc.nl*



*1053-0770/2403-0007$36.00/0*

*doi:10.1053/j.jvca.2009.10.018*

**Table 1. Composition of the Gelatin-, Albumin, and HES-Containing Cardiopulmonary Bypass Priming Solutions**

| | Volume (mL) | | |
|---|---|---|---|
| | Gelatin | Albumin | HES |
| Gelofusine | 1,000 | — | |
| Ringer's | | | 400 |
| Lactated Ringer's | 500 | 1,000 | |
| Voluven | — | — | 1,000 |
| Mannitol 20% | 100 | 200 | 100 |
| Sodium bicarbonate 8.4% | 50 | 50 | |
| Human albumin 20% | — | 300 | |
| Magnesium sulfate | — | 15 | |
| Heparin (5,000 IU/mL) | 1 | 2 | 1 |
| Volume | 1,651 | 1,567 | 1,501 |

standard vacutainer tubes (Becton Dickson, Breda, The Netherlands) containing 0.5 mL of 3.2% tri-sodium citrate. To obtain a realistic in vitro comparison, the assumption was made that the total amount of blood is commonly diluted by 30% during CPB. This dilution proportion was based on the ratio between the average male blood volume and the volume necessary to fill the disposable extracorporeal circulation system. Blood aliquots were accordingly diluted with 1 of the 3 different CPB priming solutions, and, subsequently, thromboelastometry was performed.

Three commonly used priming solutions for the extracorporeal circuit were investigated in regards to their impact on thromboelastometric variables. Priming solutions were prepared according to daily clinical routine in a collection bag, and, subsequently, a blood sample was mixed with an aliquot of this prime solution using a pipette. The composition of the 3 solutions is represented in Table 1. The 3 solutions mainly differed in the crystalloid and colloid composition. Priming solution I was based on Gelofusine (B. Braun, Oss, The Netherlands) with lactated Ringer's solution, whereas priming solution II contained Ringer's lactate with albumin (20% human albumin; Cealb Sanquin, Amsterdam, The Netherlands). Priming solution III was based on a combination of Ringer's with Voluven (Fresenius Kabi BV, Utrecht, The Netherlands).

Directly after drawing blood, baseline measurements of thromboelastometric variables were performed using rotational thromboelastometry (Rotem; Pentapharm GmbH, Munich, Germany).[18] All thromboelastometric measurements were performed at 37°C by 1 laboratory technician. Measurements were all analyzed within 2 hours after blood was drawn. After baseline measurements, blood samples were divided into 1-mL aliquots, and aliquots were subsequently diluted with priming solution in a ratio compatible with the clinical situation.

Rotem is a reagent-supported point-of-care device that provides information about the viscoelastic properties of the clot during formation and lysis. Whole blood or blood mixed with CPB priming was investigated by using 3 different analysis channels that measure and graphically display the changes in elasticity at all stages of the developing and resolving clot. Star-TEM reagent, which is a buffered, specifically concentrated calcium chloride solution to neutralize citrate, was added to all blood aliquots. (All reagents for thromboelastometry analysis [Star-TEM, ROTROL, EXTEM, INTEM, FBTEM and HEPTEM] were purchased from Pentapharm, GmbH, Munich, Germany.) To check the performance of the system, ROTROL control tests were performed weekly in accordance with the guidelines of the manufacturer. The effects of CPB priming solutions on coagulation were evaluated by using the INTEM (intrinsic coagulation pathway), the HEPTEM (similar as INTEM but replenished with heparinase), EXTEM (extrinsic coagulation pathway), and FIBTEM (fibrinogen part of the clot) tests. The INTEM was used for baseline measurements, whereas heparin-containing samples were analyzed by the HEPTEM test. For the INTEM test, ellagic acid was added to the blood mixture, which mildly activates the intrinsic coagulation pathway. The HEPTEM test is comparable with the INTEM test but contains a heparin-degrading enzyme (heparinase) and thus allows evaluation of the hemostasis in the presence of heparin. In the Results section, INTEM is used for both INTEM and HEPTEM analyses. Tissue factor from rabbit brain was added to evaluate the effects of priming on the extrinsic pathway (EXTEM). The FIBTEM test is an EXTEM-based assay that uses cytochalasin D to inhibit actin polymerization, thereby eliminating the platelet contribution of clot formation. FIBTEM is used to evaluate fibrinogen polymerization and allows the detection of fibrinogen deficiency or fibrin polymerization disorders.

Thromboelastometry provides a graph in which the strength of the clot is graphically represented over time as a characteristic cigar-shaped figure. There are several interesting hemostatic parameters that may be deduced from the thromboelastography tracing. The present study investigated the coagulation time in seconds, the maximum clot firmness in mm (MCF), and the shear elastic modulus G (dyne/cm$^2$) as a dynamic measure of clot firmness in dynes per square centimeter ($G = 5{,}000 \times MCF/[100 - MCF]$). The coagulation time represents the time from the start of the analysis until the clot starts to form. Normal coagulation times for INTEM and EXTEM are 100 to 240 seconds and 38 to 79 seconds, respectively. The MCF reflects the absolute strength of the fibrin and platelet clot and is represented by the amplitude of the cigar-shaped figure. A low MCF is indicative for disturbed hemostasis. Reference values for the MCF are 50 to 72 mm for both INTEM and EXTEM and 9 to 25 mm for FIBTEM. The shear elastic modulus (dyne/m$^2$) represents the deformability of the clot as an indicator of clot firmness and is represented by G.

Statistical tests were performed by using the SPSS statistical software package (SPSS Inc, Chicago, IL). All thromboelastometry results are expressed as mean and standard deviation (body text and tables). Statistical differences between baseline measurements and the priming solutions were analyzed by a paired-samples $t$ test. A statistical difference was reached when $p$ values were 0.05 or less.

## RESULTS

Blood samples were drawn from 8 healthy male subjects aged 31 ± 11 years. All measurements were performed in 1 blood sample per individual. Baseline thromboelastometry values are represented in Table 2 and were all within the normal range.

Blood samples were randomly diluted with the distinct priming solutions (gelatin, albumin, and HES) and further analyzed by thromboelastometry. Figure 1 shows that all priming solutions significantly increased the INTEM coagulation time from 186 ± 19 seconds (baseline) to 205 ± 16, 220 ± 17, and 223 ± 18 seconds for the gelatin-, albumin-, and HES-containing

**Table 2. Baseline Thromboelastometry Values in 8 Healthy Volunteers**

| Coagulation Parameters | |
|---|---|
| CT EXTEM (s) | 71 ± 11 |
| CT INTEM (s) | 186 ± 19 |
| MCF EXTEM (mm) | 57 ± 3 |
| MCF INTEM (mm) | 58 ± 2 |
| MCF FIBTEM (mm) | 13 ± 2 |

NOTE. Data are represented as mean ± standard deviation.