

Hooves better than potatoes: in vitro effects of balanced crystalloid and colloids on functional parameters of coagulation and fibrinolysis

Łukasz J. Krzych, Piotr F. Czempik

Department of Anesthesiology and Intensive Care, School of Medicine in Katowice, Medical University of Silesia, Katowice, Poland

Introduction Fluid resuscitation is essential to maintain or restore normovolemia during massive bleeding. Both crystalloids and colloids, by decreasing concentration of coagulation factors and number of platelets, cause dilutional coagulopathy. Additionally, synthetic colloids specifically impair polymerization of fibrin and platelet function, aggravating hemostatic disturbances.¹ Their administration may therefore increase postoperative blood loss.²

Hydroxyethyl starch (HES) affects fibrin-based clot firmness more than normal saline and Ringer's lactate solution.³ The dilutional coagulopathy produced with gelatin induces fewer coagulation abnormalities than HES.⁴ Moreover, there is a growing body of evidence that the carrier of the particles may influence hemostasis to some extent, and balanced solutions of crystalloids and colloids are considered safer than saline.⁵

We aimed to compare the in vitro effects of balanced crystalloid and 2 balanced colloids on coagulation and fibrinolysis, using rotational thromboelastometry (ROTEM).

Patients and methods The study included 32 volunteers aged 21 to 35 years (mean [SD] age, 29 [4] years), weighing 59 kg to 103 kg (mean [SD] weight, 81.2 [9.8] kg), whose physical status was deemed class I according to the American Society of Anesthesiologists. Blood samples were withdrawn from all participants. No drugs were allowed for 7 days and no alcohol or strenuous physical exercise for 1 day before blood sampling. The study was approved by the Bioethics Committee of the Medical University of Silesia in Katowice, Poland (KNW/0022/KB1/158/15/16), and written informed consent was obtained from all participants.

Sampling Venous blood samples were drawn through a 16-G or 18-G indwelling cannula

(Vasofix[®] Certo, B. Braun AG, Melsungen, Germany) from an antecubital vein of a nondominant arm. The first portion of blood (2 ml) was discarded to minimize the effect of normal saline used as a flushing solution. Subsequently, 4 blood samples were drawn: 12.5 ml at baseline and then 3 samples of 10 ml each. Dilution process was carried out immediately after blood sampling.

Hemodilution The test solutions included balanced crystalloid (Plasmalyte[®], Baxter, Poland), balanced succinylated gelatin (Geloplasma[®], Fresenius Kabi, Kutno, Poland), and balanced 6% HES 130/0.4 (Volulyte[®], Fresenius Kabi). The whole blood was diluted at a ratio of 4:1 with the study solutions to make a final concentration of 20% of each solution, equivalent of an infusion of about 1 l of a test solution to a person weighing 70 kg. Taking into account the mean (SD) weight of volunteers (81.2 [9.8] kg), the average amount of a study solution administered in this recreated scenario was 15 ml/kg. The investigator performing dilutions was not blinded to the test solutions.

Rotational thromboelastometry ROTEM coagulation analysis was carried out using a ROTEM delta analyzer (Tem Innovations GmbH, Munich, Germany), and assays were allowed to run for 30 minutes. Blood samples were analyzed immediately after sampling to minimize a preanalytical error. We performed 3 ROTEM assays simultaneously: INTEM, EXTEM, and FIBTEM. All ROTEM assays were performed by the same investigator in accordance with the manufacturer's instructions. INTEM is an intrinsic coagulation assay initiated by addition of ellagic acid. The EXTEM assay measures an extrinsic coagulation pathway by addition of tissue factor. The FIBTEM coagulation assay measures the effect of fibrin on clot firmness and uses cytochalasin D as a platelet inhibitor. The parameters measured in all 3 assays

Correspondence to:

Łukasz J. Krzych, MD, PhD,
Katedra i Klinika Anestezjologii
i Intensywnej Terapii,
Śląski Uniwersytet Medyczny
w Katowicach, ul. Medyków 14,
40-752 Katowice, Poland,
phone: +48 32 789 42 01,
e-mail: lkrzych@sum.edu.pl
Received: January 18, 2017.
Revision accepted:
February 9, 2017.
Published online: March 21, 2017.
Conflict of interests: none declared.
Pol Arch Intern Med. 2017;
127 (3): 209-211
doi:10.20452/pamw.3981
Copyright by Medycyna Praktyczna,
Kraków 2017

TABLE 1 Baseline standard laboratory coagulation tests

Parameter	Mean (SD)	Median (interquartile range)	Reference value
aPTT, s	30.22 (4.05)	29.95 (27.7–31.95)	25–37
PT, s	12.45 (0.79)	12.5 (11.75–13.05)	10.4–13.0
INR	1.05 (0.07)	1.05 (0.99–1.1)	0.9–1.2
Fibrinogen, mg/dl	246.97 (31.78)	235.0 (212.99–250.07)	210–400
D-dimer, µg/ml	0.20 (0.08)	0.17 (0.17–0.19)	0–0.50
Platelets, 10 ⁹ /µl	228.41 (36.89)	222.0 (175.99–237.0)	150–350

Abbreviations: aPTT, activated partial thromboplastin time; INR, international normalized ratio; PT, prothrombin time

included coagulation time (CT; reference value, 100–240 s [INTEM], 38–79 s [EXTEM]), clot formation [CF] time (CFT; reference value: 30–110 s [INTEM], 34–159 s [EXTEM]), CF ratio (CFR; no reference value given), alpha angle (AA; reference value, 70°–83° [INTEM], 63°–83° [EXTEM]), amplitude at different time points (minutes) (eg, A10; reference value, 44–66 mm [INTEM], 43–65 mm [EXTEM], 7–23 mm [FIBTEM]), maximum clot firmness (MCF; reference value, 50–72 mm [INTEM, EXTEM]), actual clot firmness (ACF; no reference value given), maximum lysis (ML; reference value, <15% [INTEM, EXTEM]), and area under the clotting curve at different time points (minutes) (AR5, AR10, AR15, AR20, AR25; no reference values established). MCF is the maximum firmness of the clot and is influenced by fibrin, platelets, and factor XIII.

Statistical analysis The statistical analysis was performed using the MedCalc statistical software (Version 16.1 2016, MedCalc Software bvba, Belgium). Demographic characteristics were shown as a mean (SD). All ROTEM values were presented as a median and interquartile range. The differences between undiluted and diluted samples were assessed using tests for repeated measures (repeated analysis of variance or Friedman test). The level of statistical significance was set at a *P* value of less than 0.05.

Results The baseline results of standard laboratory tests of coagulation are provided in **TABLE 1**. The effects of all investigated fluids on coagulation are presented in Supplementary material online, *Table S1*. Plasmalyte® had no impact on ex vivo hemostasis. Significant dilution was observed only for colloids, and Geloplasma® produced fewer coagulation abnormalities than HES. In INTEM, we found impaired CF (measured by CFT and AA) as well as clot strength (measured by A10, A15, A20, and MCF). FIBTEM and EXTEM CT fell outside the reference values only for HES. Other parameters reflecting fibrinogen contribution to clot strength were also impaired only by HES. No test solution impacted EXTEM parameters of clot firmness (ie, A10, A15, A20, and MCF). No effect on fibrinolysis (as evidenced by ML) in INTEM and EXTEM was noticed for all solutions.

Discussion Our ex vivo study showed a minor effect of the balanced crystalloid at hemodilution of 20%. Dilution with gelatin impaired coagulation to a lesser extent than HES. The most pronounced effect of HES was the decrease in fibrinogen levels and impaired fibrin polymerization. Fibrinolysis was undisturbed by all balanced solutions.

We decided to implement the ROTEM analysis as the most reliable method of hemostasis assessment. Three assays were used: INTEM, EXTEM, and FIBTEM. INTEM is dependent on coagulation factors (XII, XI, IX, VIII, X, V, II, I), platelets, and fibrinolysis. Contributors to EXTEM are coagulation factors (VII, X, V, II, I), platelets, and fibrinolysis. Finally, FIBTEM is carried out to perform differential diagnosis between fibrinogen depletion, fibrinogen dysfunction, and platelet dysfunction. Fibrinogen is an essential precursor of CF.

In our in vitro study, dilution with balanced medium-molecular-weight HES with low degree of substitution (ie, 0.4) impaired hemostasis to a higher extent than balanced gelatin. This effect was demonstrated by deranged parameters obtained with 3 ROTEM assays. Using INTEM, we showed decreased CF (CFT, AA) and clot firmness (amplitude at different time points, MCF) induced by Volulyte® and Geloplasma®, although the effect for Volulyte® was more pronounced. In the FIBTEM assay, CF and clot strength was impaired exclusively by Volulyte®.

The clinical coagulopathic effects are mostly seen when large volume (>1.5 l) of colloids have been infused or in patients with previous coagulopathy.⁶ In our study, we used moderate dosing of HES. The real novelty of our findings is that, although we used the dose of HES that is recommended by the manufacturer, we still showed negative impact of this solution on hemostasis. Our findings therefore support the recommendation against the use of HES in this context.

According to previous studies, dilution with gelatin reduces CF to a lesser degree than HES.⁷ Several in vitro and in vivo studies have demonstrated that gelatin solution has only moderate effects on the hemostatic process as compared to other colloids. It is noteworthy that the effects of gelatin on coagulation are almost completely reversed by increasing the concentration of fibrinogen.⁴

Our results showing an insignificant effect of crystalloid on hemostasis are also comparable

with previous studies. Only at high dilution, the effect of balanced crystalloid like Ringer's lactate solution might be detected.⁹ In our study, the effect of balanced crystalloid (Plasmalyte®) on coagulation was minor, possibly due to limited dilution (20%).

The effects of HES on fibrinolysis have also been studied with thromboelastometry and confirmed the negative effect only after dilution with HES.⁹ In our study, no test solution impacted fibrinolysis.

An interesting finding of our study is the possible impact of fluids used for priming of the cardiopulmonary bypass system in cardiac surgery on perioperative blood loss. Up to 15% of the procedures requiring cardiopulmonary bypass are complicated with postoperative hemorrhage; thus, cardiac surgery is a discipline that consumes the majority of blood products. Therefore, taking into account the results of our study, the use of HES as a priming solution is controversial. On the contrary, a recent analysis showed that the safety profile of gelatin and HES as a priming solution is comparable to that of crystalloids,¹⁰ so we need more data to draw definite conclusions.

Our study has limitations of an in vitro study. First of all, our findings may differ from those observed for in vivo fluid resuscitation, including changes in the coagulation system, response to tissue and endothelial injuries, as well as changes in kinetics, volume of distribution, or acid-base balance. On the one hand, in vivo studies deliver more physiological data. However, the findings in healthy volunteers also differ from those in the scenario of massive bleeding in real coagulopathy. Secondly, one ought to remember that only a 20% dilution was made, which may be insufficient to observe coagulation abnormalities, even with the ROTEM analysis.

In conclusion, fluid resuscitation with balanced crystalloid solutions causing the 20% blood dilution seems safe in terms of interference with coagulation and fibrinolysis. Gelatins may be a reasonable choice for a second-line treatment when further volume expansion is required. HES should rather be avoided due to its detrimental effect on hemostasis, even in doses recommended by the manufacturer.

Supplementary material online Supplementary material online is available with the online version of the article at www.pamw.pl.

Acknowledgments The study was supported by the 2015 annual grant of the Polish Cardiac Society funded by Adamed corporation (to ŁK). We express our gratitude to the personnel of the central hospital laboratory in the Silesian Centre for Heart Diseases in Zabrze for their voluntary extra work providing determinations for the study.

REFERENCES

- 1 Nielsen VG. Colloids decrease clot propagation and strength: role of factor XIII-fibrin polymer and thrombin-fibrinogen interactions. *Acta Anaesthesiol Scand.* 2005; 49: 1163-1171.
- 2 Lee JW, Kweon TD, Kwak YL, et al. The effect of 6% HES 130/0.4 on hemostasis and hemodynamic efficacy in off-pump coronary artery bypass surgery: a comparison with 6% HES 200/0.5 Korean J Anesthesiol. 2007; 53: 14-21.
- 3 Godier A, Durand M, Smadja D, et al. Maize- or potato-derived hydroxyethyl starches: is there any thromboelastometric difference? *Acta Anaesthesiol Scand.* 2010; 54: 1241-1247.
- 4 Fries D, Innerhofer P, Klingler A, et al. The effect of combined administration of colloids and lactated Ringer's solution on the coagulation system: An in vitro study using thrombelastograph coagulation analysis (ROTEG1). *Anesth Analg.* 2002; 94: 1280-1287.
- 5 Verma B, Luethi N, Cioccarli L, et al. A multicentre randomised controlled pilot study of fluid resuscitation with saline or Plasma-Lyte 148 in critically ill patients. *Crit Care Resusc.* 2016; 18: 205-212.
- 6 Levi M, Jonge Ed. Clinical relevance of the effects of plasma expanders on coagulation. *Semin Thromb Hemost.* 2007; 33: 810-815.
- 7 Petroianu GA, Liu J, Maleck WH, et al. The effect of In vitro hemodilution with gelatin, dextran, hydroxyethyl starch, or Ringer's solution on thrombelastograph. *Anesth Analg.* 2000; 90: 795-800.
- 8 Konrad CJ, Markl TJ, Schuepfer GK, et al. In vitro effects of different medium molecular hydroxyethyl starch solutions and lactated Ringer's solution on coagulation using SONOCLOT. *Anesth Analg.* 2000; 90: 274-279.
- 9 Egli GA, Zollinger A, Seifert B, et al. Effect of progressive haemodilution with hydroxyethyl starch, gelatin and albumin on blood coagulation. *Br J Anaesth.* 1997; 78: 684-689.
- 10 Ghijssels I, Himpe D, Rex S. Safety of gelatin solutions for the priming of cardiopulmonary bypass in cardiac surgery: a systematic review and meta-analysis. *Perfusion.* 2016; 1: 267659116685418. doi:10.1177/0267659116685418