Rev Bras Anestesiol. 2019;69(4):383–389

REVISTA BRASILEIRA DE ANESTESIOLOGIA
Publicação Oficial da Sociedade Brasileira de Anestesiologia
www.sba.com.br

SCIENTIFIC ARTICLE

Rotational thromboelastometry assessment of ballanced crystalloid, hydroxyethyl starch and gelatin effects on coagulation: a randomized trial

Silvie Sevcikova, Miroslav Durila *, Tomas Vymazal

Charles University and Motol University Hospital, Second Faculty of Medicine, Department of Anaesthesiology and Intensive Care Medicine, Prague, Czech Republic

Received 19 October 2018; accepted 17 March 2019
Available online 22 May 2019

KEYWORDS
Arthroscopy;
Coagulopathy;
Crystalloids;
Gelatin;
Hydroxyethyl starch;
Rotational thromboelastometry

Abstract
Background and objectives: Modern crystalloid and colloid solutions are balanced solutions which are increasingly used in perioperative period. However, studies investigating their negative effect on whole blood coagulation are missing, and vivid debate is going on about which solution has the minimal coagulopathy effect. The aim of our study was to assess the effect of modern fluid solutions on whole blood coagulation using rotational thromboelastometry.
Methods: Blood samples were obtained from 30 patients during knee arthroscopy before and after administration of 500 mL of crystalloid, Hydroxyethyl Starch and gelatin according to the randomization. Rotational thromboelastometry (Extem, Intem and Fibtem tests) was used to assess negative effect of fluid solutions on whole blood coagulation.
Results: In Extem test, the initiation phase of fibrin clot formation represented by CT parameter was not influenced by any fluid solution ($p > 0.05$). The speed of clot formation represented by CFT and α angle was impaired by Hydroxyethyl Starch and gelatin but not by crystalloids ($p < 0.05$). The strength of formatted coagulum represented by MCF parameter was impaired both in Extem and Fibtem test by HES and in Fibtem also by crystalloids ($p < 0.05$). Intem test was not negatively influenced by any crystalloid or colloid solution in any parameter ($p > 0.05$).
Conclusion: Extem test appears to be sensitive to coagulopathy effect of modern colloids and crystalloids. Hydroxyethyl starch has the most obvious negative effect on clot formation followed by gelatin and finally by crystalloids. Intem test seems to be insensitive to adverse effect of modern colloids and crystalloids.

* Corresponding author.
E-mail: miroslav.durila@fnmotol.cz (M. Durila).

https://doi.org/10.1016/j.bjane.2019.04.007

PALAVRAS-CHAVE
Artroscopia;
Coagulopatia;
Cristaloides;
Gelatina;
Hidroxietilamido;
Tromboelastometria rotacional

Tromboelastometria rotacional na avaliação dos efeitos de cristaloides balanceados, hidroxietilamido e gelatina na coagulação: estudo randômico

Resumo
Justificativa e objetivos: Os cristaloides e coloides modernos são soluções balanceadas e cada vez mais utilizadas no período perioperatório. No entanto, não há estudos que avaliem seu efeito negativo na coagulação do sangue total e o intenso debate sobre a solução que cause um efeito mínimo na coagulopatia permanece. O objetivo de nosso estudo foi avaliar o efeito das soluções líquidas modernas na coagulação do sangue total com o uso da tromboelastometria rotacional.
Métodos: De acordo com a randomização, amostras de sangue foram colhidas de 30 pacientes durante a artroscopia de joelho, antes e após a administração de 500 mL de cristaloides, hidroxietilamido e gelatina. A tromboelastometria rotacional (testes Extem, Intem e Fibtem) foi utilizada para avaliar o efeito negativo das soluções líquidas na coagulação do sangue total.
Resultados: No teste Extem, a fase de iniciação da formação de coágulos de fibrina representada pelo parâmetro CT não foi influenciada por nenhuma solução líquida ($p > 0,05$). A velocidade da formação de coágulos representada pelo CFT e pelo ângulo α foi prejudicada pelo hidroxietilamido e pela gelatina, mas não pelos cristaloides ($p < 0,05$). A força do coágulo formatado representado pelo parâmetro MCF foi prejudicada tanto no teste Extem quanto no teste Fibtem pelo HES e no teste Fibtem também pelos cristaloides ($p < 0,05$). O teste Intem não foi influenciado negativamente por nenhuma solução cristaloide ou coloide em nenhum parâmetro ($p > 0,05$).
Conclusão: O teste Extem parece ser sensível ao efeito de coagulopatia dos coloides e cristaloides modernos. O hidroxietilamido apresentou o efeito negativo mais óbvio na formação do coágulo, seguido pela gelatina e finalmente pelos cristaloides. O teste Intem parece ser insensível ao efeito adverso dos coloides e cristaloides modernos.

Introduction

Crystalloid and colloid solutions are used to maintain adequate tissue perfusion during perioperative period as well as during treatment of any kind of shock in critically ill patients. A variety of crystalloids and colloids are used in clinical practice. Basic saline solution has been used for over 50 years in a multitude of clinical situations despite its unphysiological properties[1,2] and from unbalanced colloids Hydroxyethyl Starch (HES) and gelatin are used very often. Recently, modern crystalloid and colloid solutions called balanced (more physiologic) solutions with a chemical composition that approximates extracellular space, are increasingly used in clinical practice and according to published studies they seem to be more beneficial for majority of patients comparing to unbalanced fluid solutions.[2–4] They contain high molecular weight substances and under optimal condition, especially when the glycocalyx is intact, they remain in circulation for longer time than crystalloids.[1–5] According to previous studies, unbalanced colloids HES and gelatin alter haemostasis to some degree but data on balanced colloids are missing.[6–9] As it is still frequently discussed which type of solution should be preferred in the perioperative care and life threatening bleeding, the study investigating coagulopathy effect of balanced fluid solutions is needed.

Rotational Thromboelastometry (ROTEM) is a bed-side whole blood coagulation test which evaluates viscoelastic properties of formed coagulum during all phases of coagulation such as initiation, propagation (the speed of clot formation), strength of coagulum and stability of the clot represented by fibrinolysis.[10] Comparing to plasma coagulation tests such as Prothrombin Time (PT) and Activated Thromboplastin Time (aPTT), ROTEM provides results within 10 min from initiation of the test, which is critical in situations of life threatening bleeding in operating room or in trauma patients. ROTEM has been included in recent European guidelines for perioperative and trauma bleeding management by the European Society of Anaesthesiologists.[11,12]

In our study we decided to investigate the coagulopathy effect of modern balanced crystalloid and colloid solutions using ROTEM *in vivo*. The aim of this study was to find the fluid solution with minimal negative effect on whole blood coagulation assessed by ROTEM.

Methods

The project was approved by the Ethics Committee for Multi-Centric Clinical Trials of the University Hospital Motol and informed consent was obtained from all participants. As in human studies it is very challenging to investigate large

number of patients we decided to enrol to study and obtain blood samples from 30 healthy patients who were undergoing knee arthroscopy (aged 44 ± 7 years; body mass index 25 ± 2.5; 15 males, 15 females; no comorbidities; ASA 1 – according to classification of American Society of Anaesthesiologists).

All patients were non-smokers, not taking any drugs influencing coagulation (contraceptive, anticoagulant or antiplatelet therapy) and without history of any coagulopathy disorder. Before the induction of general anaesthesia, 2 mL of venous blood were obtained from patients into 2 mL tube containing 0.105 M of sodium citrate with citrate concentration of 3.2%. This sample was used as a control and following thromboelastometry tests were performed: EXTEM for analysing extrinsic pathway of coagulation (contains activator of extrinsic pathway of coagulation), INTEM for analysing intrinsic pathway of coagulation (contains activator of intrinsic pathway of coagulation), FIBTEM for assessment of functional fibrinogen level (contains antiplatelet substance). The following ROTEM parameters were evaluated: CT – clotting time, representing initiation phase of coagulation (time to first detectable fibrin formation), α angle and CFT – clot formation time, both representing propagation phase of coagulation, MCF – maximum clot firmness. To evaluate platelet function, according to the authors Solomon et al., the difference between values of EXTEM MCF (depends on level of fibrinogen and platelet function) and FIBTEM MCF (depends on fibrinogen level) was calculated and derived as parameter MCE (maximum clot elasticity attributable to platelets, parameter representing platelets function and their component in EXTEM MCF).[13]

In clinical practice the drop of blood pressure after induction of general anaesthesia is usually treated by administering some fluid solution depending on preferences of the anaesthetist. In our study the type of fluid was chosen according to randomization of patients into three equal groups using generator of random numbers in spread sheet. The first group of patients was infused 500 mL of balanced crystalloid Plasma-Lyte (isotonic, BAXTER, USA). The second group was infused 500 mL of balanced colloid Volulyte 6% representing hydroxyethyl starch (130/0.4, isotonic, FRESENIUS KABI, Germany) and the third group was infused 500 mL of balanced colloid Gelaspan 4% representing gelatin solution (isotonic, B BRAUN, Germany). Described volume of 500 mL was chosen as it is routinely used in daily practice to treat arterial hypotension induced by anaesthetic drugs. Fifteen minutes after the solution was completely infused into the patient another blood sample was obtained and thromboelastometry basic tests EXTEM, INTEM and FIBTEM were performed again to identify presence of any coagulation changes as compared to the control samples.

To rule out potential bias caused by different dilution effect of different types of solution on coagulation (as it is generally believed that colloids stay longer in blood vessels comparing to crystalloids), haemoglobin level was measured before and after infusion simultaneously with blood sampling for ROTEM.

Statistical analysis

For data analysis the GraphPad Prism 6.0 statistics program was used. Nonparametric Wilcoxon matched-pairs signet rank test was used for analyses of ROTEM parameters. For haemoglobin measurement comparing decrease in haemoglobin in percentage after fluid administration among all three types of fluid solution nonparametric Kruskal–Wallis test of one-way ANOVA was used. Statistical significance was described as two-tailed p-value <0.05.

Results

When investigating extrinsic pathway of coagulation using EXTEM test, no statistically significant adverse effect of crystalloids or HES/gelatin on initiation phase of fibrin clot formation represented by CT parameter was observed ($p > 0.05$) (Fig. 1A–C). However, kinetics represented by CFT and α angle was negatively influenced by HES and gelatin ($p < 0.05$) but not by crystalloids (Fig. 1D–I). Strength of formatted coagulum represented by MCF parameter was significantly influenced only by HES ($p > 0.05$) (Fig. 2A–C).

Functional fibrinogen level represented by MCF FIBTEM was significantly decreased by crystalloids and HES ($p < 0.05$) but not by gelatin (Fig. 2D–F). Parameter MCE representing platelet function was not significantly influenced by any fluid solution (Fig. 2G–I).

Intrinsic pathway of coagulation assessed by INTEM was not negatively influenced either by crystalloids or HES/Gelatin in any parameter ($p > 0.05$) (Table 1).

There was no statistically significant difference in decrease of haemoglobin level after infusion of all types of fluids ($p < 0.05$) (Fig. 3).

Discussion

The type of fluid solution used during perioperative period is still widely discussed, especially its potential coagulopathy effect. In our study we investigated effect of modern balanced fluids on whole blood coagulation assessed by ROTEM, as they are more frequently used in clinical practice nowadays and there are missing data about their effect on coagulation. According to our results, initiation phase of clot formation in extrinsic and intrinsic pathway is not influenced by any type of administered fluid. However, propagation phase was negatively influenced by both HES and gelatin, while strength of coagulum was impaired only by HES. Authors Rasmussen et al. in their extensive meta-analysis of perioperative fluid therapy concerning coagulopathy similarly describe that administration of HES is followed by weakening of coagulum indicated by decreasing parameter of Maximal Amplitude (MA) measured by thrombelastography.[14] Our findings also partially correspond with study of Sawhney et al. who described that infusion of 1 L of HES to traumatic patients impairs coagulation more than gelatine.[15] Schlimp with Cadamuro describe that HES as well as gelatin decreased maximum clot firmness of both EXTEM and FIBTEM.[7] However, we did not see a similar

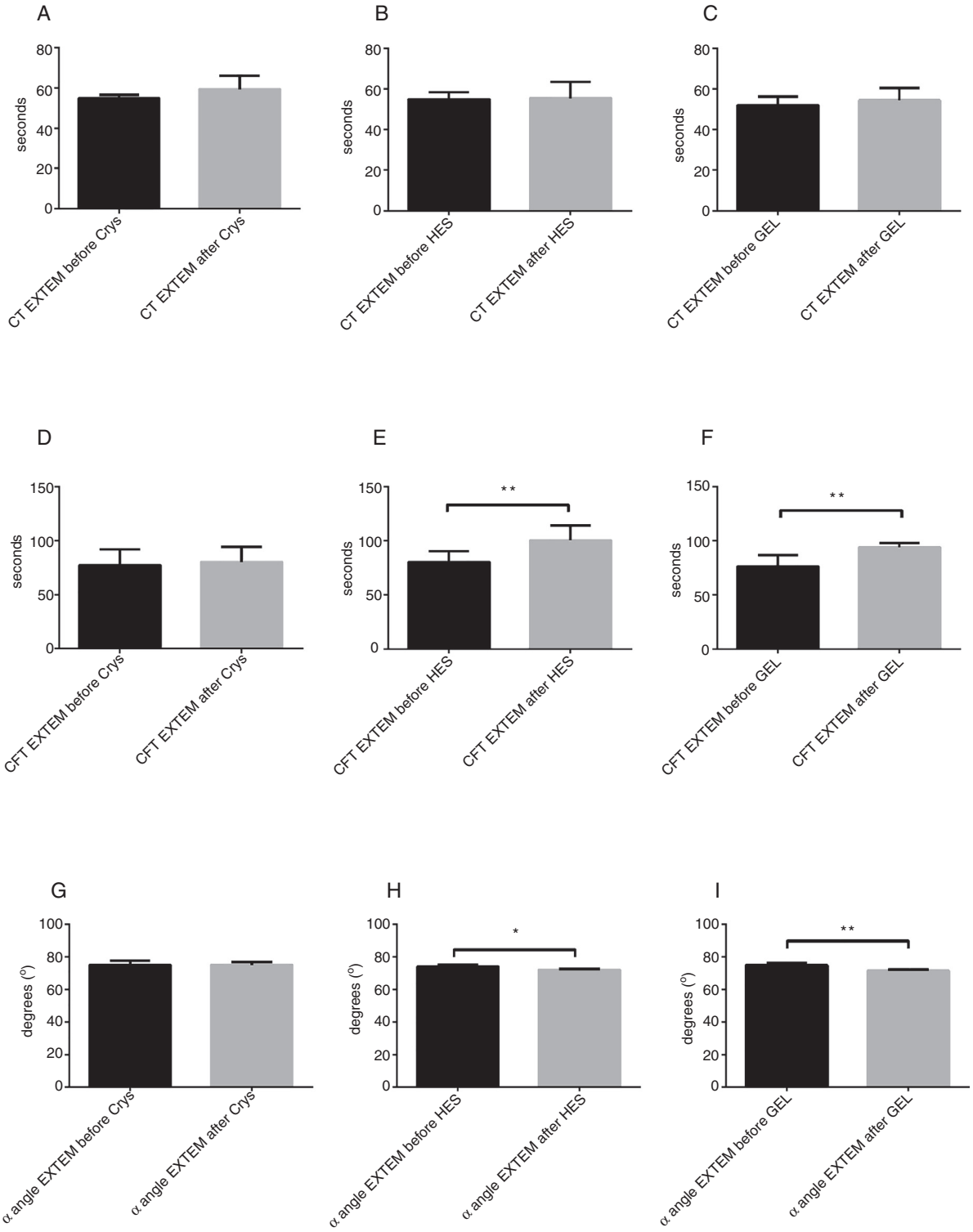

Figure 1 EXTEM parameters describing initiation and propagation of clot formation before and after fluid infusion (medians with interquartile range). (A) CT, Clotting Time (representing initiation of clot formation) in Crystalloid Group (Crys); (B) CT in Hydroxyethyl Starch Group (HES); (C) CT in Gelatin Group (GEL); (D) CFT, Clot Formation Time (representing propagation – speed of clot formation) in Crystalloid Group (Crys); (E) CFT in Hydroxyethyl Starch Group (HES); (F) CFT in Gelatin Group (GEL); (G) α angle (representing propagation – speed of clot formation) in Crystalloid Group (Crys); (H) α angle in Hydroxyethyl Starch Group (HES); (I) α angle in Gelatin Group (GEL).

* Means significant difference $p < 0.05$.

** Means significant difference $p < 0.01$.

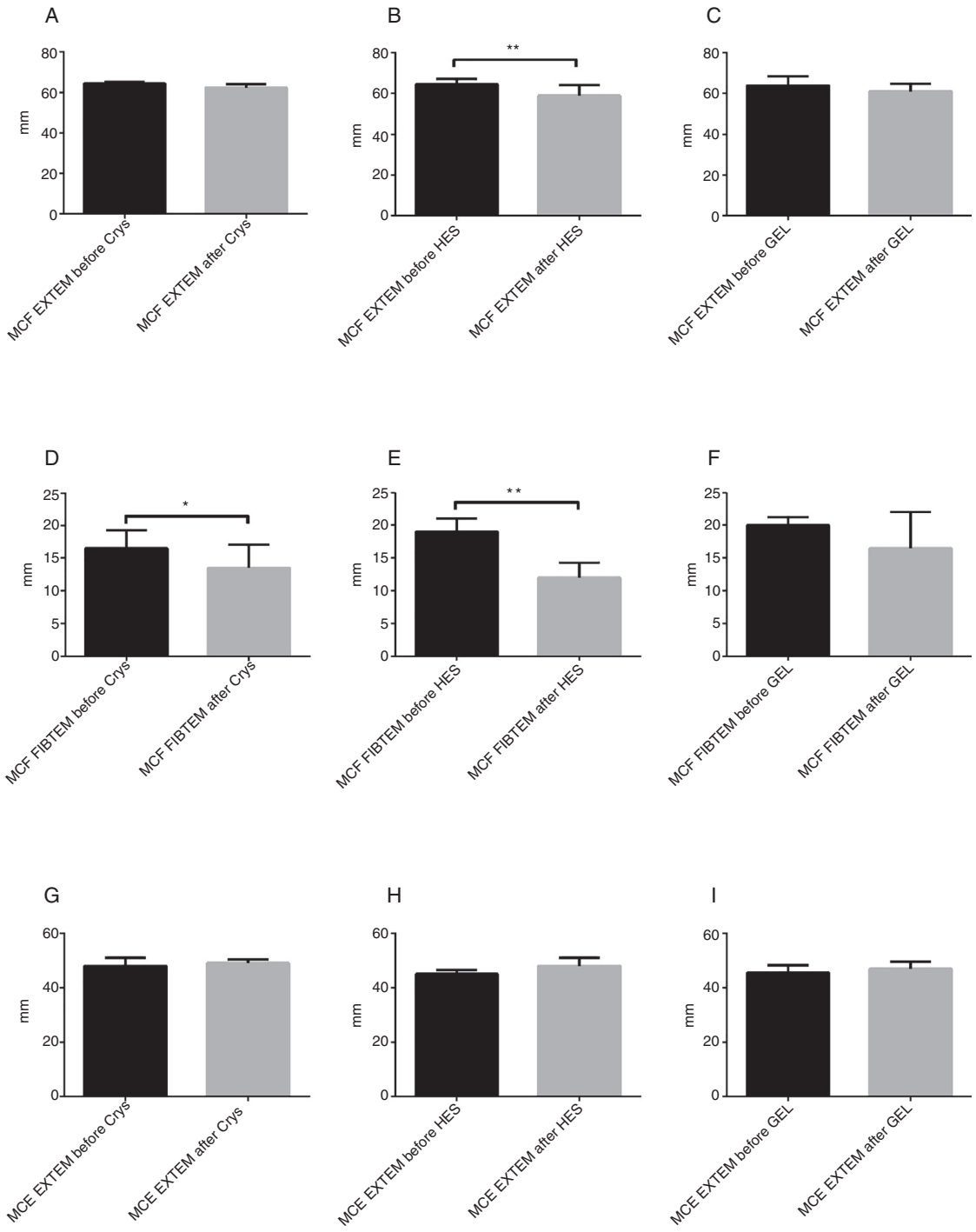

Figure 2 EXTEM and FIBTEM parameters describing maximal clot strength and platelets function before and after fluid infusion (medians with interquartile range). (A) MCF EXTEM, maximum clot firmness (representing maximal clot strength) in Crystalloid Group (Crys); (B) MCF EXTEM in Hydroxyethyl Starch Group (HES); (C) MCF EXTEM in Gelatin Group (GEL); (D) MCF FIBTEM (representing maximal clot strength of coagulum after blocking platelet function, functional fibrinogen level) in Crystalloid Group (Crys); (E) MCF FIBTEM in Hydroxyethyl Starch Group (HES); (F) MCF FIBTEM in Gelatin Group (GEL); (G) MCE EXTEM, maximum clot elasticity of EXTEM (difference between MCF EXTEM and MCF FIBTEM, represents platelet function) in Crystalloid Group (Crys); (H) MCE EXTEM in Hydroxyethyl Starch Group (HES); (I) MCF EXTEM in Gelatin Group (GEL).

* Means significant difference $p < 0.05$.

** Means significant difference $p < 0.01$.

Table 1 Non-significant differences of mean ± SD of INTEM parameters obtained before and after fluid infusion.

INTEM	CT (s)		CFT (s)		α angle (°)		MCF (mm)	
	Mean ± SD before	Mean ± SD after	Mean ± SD before	Mean ± SD after	Mean ± SD before	Mean ± SD after	Mean ± SD before	Mean ± SD after
Crystalloid	169 ± 16	159 ± 28	84 ± 23	80 ± 24	73 ± 4	74 ± 5	60 ± 3	60 ± 4
HES	165 ± 13	152 ± 12	83 ± 29	86 ± 16	74 ± 4	74 ± 2	58 ± 6	56 ± 5
GEL	171 ± 27	142 ± 34	73 ± 13	78 ± 13	75 ± 3	74 ± 3	60 ± 4	60 ± 5

CT, Clotting Time (representing initiation of clot formation); CFT, Clot Formation Time; α angle, representing propagation – speed of clot formation; MCF, Maximum Clot Firmness (representing maximal clot strength); HES, Hydroxyethyl Starch; GEL, Gelatin; SD, standard deviation; INTEM, intrinsic pathway of coagulation.

coagulopathy effect of gelatin when using only 500 mL volume – this volume did probably not produce dilutional effect on coagulation. The phenomenon can be explained by using modern balanced solution of gelatin (more physiologic) in our study, using smaller volume and by performing this study *in vivo*, not *in vitro* as the other authors did. Again, authors Mauch and Madjdpour et al. described that HES and gelatin impair blood coagulation although they did not notice significant difference between HES and gelatin, but again they used unbalanced fluids and the study was performed on piglets.[16] On the other hand, we have recently published an *in vitro* study where the negative effect of HES and gelatin on coagulation was also found, but dilution of blood sample was higher of about 20%.[17] We may assume that when the higher fluid volume is used, the higher disturbance of coagulation may be present.

The crystalloid solution did not cause hypocoagulation in any of EXTEM and INTEM parameters despite decrease of FIBTEM MCF parameter. Neither authors Schlimp and Cadamuro found hypocoagulation effect of crystalloids, moreover they described reduced clotting time.[7] Similarly, Sawhney et al. describe hypercoagulation effect of crystalloid after infusion of 1 L in trauma patients.[15] Ponschab et al. describe impaired blood coagulation measured by ROTEM in pigs, represented by worsened propagation phase of fibrin formation and strength of coagulum, when high volume of either 1 L or 3 L was infused.[18] Therefore, it seems that the crystalloid effect on coagulation depends on infusion volume and type of solutions.

It would be ideal to use power analysis for calculation of appropriate number of investigations needed to predict significant differences in results; however, it was not possible to do it for our study because similar studies are missing. Moreover, in human studies it is very challenging to investigate large numbers of patients. We decided to include 30 patients (10 in each group) and use nonparametric Wilcoxon matched-pairs signet rank test for analyses of ROTEM parameters. Statistically significant results were found in results described by two-tailed p-value <0.05. After finding significant differences in our study using the described method and p-value, the results can be assessed as reliable despite relatively low number of samples investigated. Therefore, power analysis is not needed anymore.

The last but not the least thing that has to be discussed, is the question whether coagulopathy effect of fluids observed in our study was not caused by a simple dilutional effect of volume infused to the patient. Therefore, haemoglobin level was measured before and again after infusion. Interestingly, all types of fluid solutions decreased haemoglobin level in similar way and there was no statistically significant difference among percentage decrease in its level among all three groups. This means that all three types of fluid solution have produced dilution of similar degree and thus, similar degree of dilutional coagulopathy would be expected in all investigated groups. As our results do not seem to show that, we can consider findings of our study reliable.

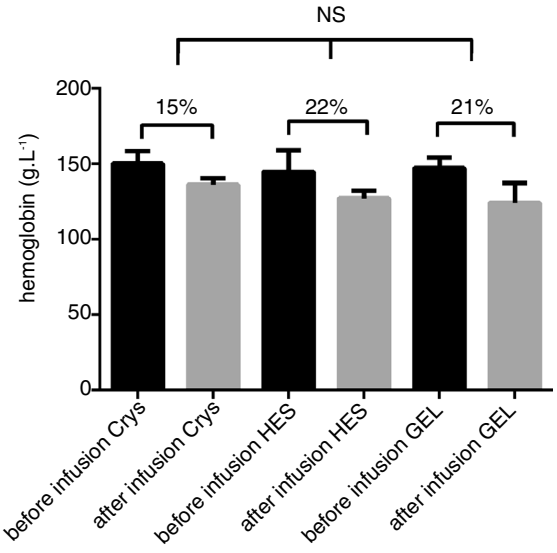

Figure 3 Haemoglobin levels before and after infusion of different type of fluid (medians with interquartile range). Haemoglobin level before and after infusion of Crystalloid (Crys), Hydroxyethyl Starch (HES) and Gelatin (GEL); %, describes decrease of haemoglobin before and after infusion of fluid solution in percentage; NS, Non-Significant difference among decrease of haemoglobin level ($p > 0.05$).

The limitation of this study may seem to be the fact that we evaluated blood coagulation in patients undergoing relatively small surgical procedure which is not usually accompanied with great blood loss. However, we believe that in low risk bleeding procedures of this type we can assess the direct coagulopathy effect of crystalloids and colloids more reliably comparing to high risk bleeding procedures. On the other hand, in a situation of huge blood loss, it can be expected that simple volume replacement would cause dilution of coagulation factors leading to

coagulopathy, regardless of the type of administered solution. In addition, different types of fluid solutions might aggravate already induced coagulopathy.

Conclusion

EXTEM test representing the extrinsic pathway of coagulation seems to be more sensitive to coagulopathy effect of modern balanced colloids and crystalloids. In our study hydroxyethyl starch had the most obvious negative effect on clot formation followed by gelatin and finally by crystalloids. INTEM test representing the intrinsic pathway of coagulation was not negatively influenced by any colloid or crystalloid solution.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

This study was supported by projects of Ministry of Health for conceptual development Research organization (no. 00064203) Motol Hospital.

References

1. Myburgh JA, Mythen MG. Resuscitation fluids. N Engl J Med. 2013;369:2462–3.
2. Allen SJ. Fluid therapy and outcome: balance is best. J Extra Corpor Technol. 2014;46:28–32.
3. Kuca T, Butler MB, Erdogan M, et al. A comparison of balanced and unbalanced crystalloid solutions in surgery patient outcomes. Anaesth Crit Care Pain Med. 2017;36:371–6.
4. Self WH, Semler MW, Wanderer JP, et al. Saline versus balanced crystalloids for intravenous fluid therapy in the emergency department: study protocol for a cluster-randomized, multiple-crossover trial. Trials. 2017;18:178.
5. Marx G, Schuerholz T. Fluid-induced coagulopathy: does the type of fluid make a difference? Crit Care. 2010;14:118.
6. Shin HJ, Na HS, Do SH. The effects of acute normovolaemic haemodilution on peri-operative coagulation in total hip arthroplasty. Anaesthesia. 2015;70:304–9.
7. Schlimp CJ, Cadamuro J, Solomon C, et al. The effect of fibrinogen concentrate and factor XIII on thromboelastometry in 33% diluted blood with albumin, gelatine, hydroxyethyl starch or saline in vitro. Blood Transfus. 2013;11:510–7.
8. Lobo DN, Stanga Z, Aloysius MM, et al. Effect of volume loading with 1 liter intravenous infusions of 0.9% saline, 4% succinylated gelatine (Gelofusine) and 6% hydroxyethyl starch (Voluven) on blood volume and endocrine responses: a randomized, three-way crossover study in healthy volunteers. Crit Care Med. 2010;38:464–70.
9. Thomas-Rueddel DO, Vlasakov V, Reinhart K, et al. Safety of gelatin for volume resuscitation – a systematic review and meta-analysis. Intensive Care Med. 2012;38:1134–42.
10. Veigas PV, Callum J, Rizoli S, et al. A systematic review on the rotational thrombelastometry (ROTEM(R)) values for the diagnosis of coagulopathy, prediction and guidance of blood transfusion and prediction of mortality in trauma patients. Scand J Trauma Resusc Emerg Med. 2016;24:114.
11. Kozek-Langenecker SA, Ahmed AB, Afshari A, et al. Management of severe perioperative bleeding: guidelines from the European Society of Anaesthesiology: first update 2016. Eur J Anaesthesiol. 2017;34:332–95.
12. Rossaint R, Bouillon B, Cerny V, et al. The European guideline on management of major bleeding and coagulopathy following trauma: fourth edition. Crit Care. 2016;20:100.
13. Solomon C, Ranucci M, Hochleitner G, et al. Assessing the methodology for calculating platelet contribution to clot strength (platelet component) in thromboelastometry and thrombelastography. Anesth Analg. 2015;121:868–78.
14. Rasmussen KC, Secher NH, Pedersen T. Effect of perioperative crystalloid or colloid fluid therapy on hemorrhage, coagulation competence, and outcome: a systematic review and stratified meta-analysis. Medicine (Baltimore). 2016;95:e4498.
15. Sawhney C, Subramanian A, Kaur M, et al. Assessment of hemostatic changes after crystalloid and colloid fluid preloading in trauma patients using standard coagulation parameters and thromboelastography. Saudi J Anaesth. 2013;7:48–56.
16. Mauch J, Madjdpour C, Kutter AP, et al. Effect of rapid fluid resuscitation using crystalloids or colloids on hemostasis in piglets. Paediatr Anaesth. 2013;23:258–64.
17. Sevcikova S, Vymazal T, Durila M. Effect of balanced crystalloid, gelatin and hydroxyethyl starch on coagulation detected by rotational thromboelastometry in vitro. Clin Lab. 2017;63:1691–700.
18. Ponschab M, Schochl H, Keibl C, et al. Preferential effects of low volume versus high volume replacement with crystalloid fluid in a hemorrhagic shock model in pigs. BMC Anesthesiol. 2015;15:133.