

Effect of Selected Crystalloid and Colloid Solutions on Coagulation Status Evaluated by Rotational Thromboelastometry and Platelet Function Analyser: An in vitro Study

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Keywords

Colloids and crystalloids · Haemocoagulation · Platelet function analysis · Rotational thromboelastometry

Abstract

Introduction: Crystalloid and colloid solutions commonly used in intensive and perioperative care can affect haemocoagulation status. This in vitro study assessed the impact of Plasma-Lyte, albumorm 5%, and Gelaspan 4% solutions on primary and secondary haemostasis using rotational thromboelastometry and platelet function analyser.

Methods: In this prospective study, we examined blood samples from 20 healthy volunteers using rotational thromboelastometry and platelet function analyser. Simultaneously, we analysed the blood samples subjected to 10% dilution using Plasma-Lyte, albumorm 5%, and Gelaspan 4% solutions. **Results:** Compared to controls, Plasma-Lyte shortened EXTEM-CT ($p = 0.005$) and reduced FIBTEM-MCF ($p = 0.017$). albumorm 5% prolonged EXTEM-CFT ($p = 0.001$), decreased EXTEM-alpha ($p < 0.001$) and MCF in EXTEM, INTEM, and FIBTEM tests ($p < 0.001$, $p = 0.038$, $p = 0.001$, respectively), along with MCE in the PLTEM test ($p < 0.001$). Gelaspan 4% also prolonged EXTEM-CFT ($p < 0.001$), decreased EXTEM-alpha ($p < 0.001$) and MCF in EXTEM, INTEM, and FIBTEM tests ($p < 0.001$, $p < 0.001$, $p = 0.009$, respectively), along with MCE in the PLTEM test ($p < 0.001$). Gelaspan 4% also reduced EXTEM-CT ($p = 0.021$). All solution prolonged CT in PFA Col/ADP ($p = 0.003$ for Plasma-Lyte, $p < 0.001$ for albumorm and Gelaspan) and albumorm 5% also

prolonged CT in Col/Epi ($p = 0.003$). **Conclusion:** Plasma-Lyte had the least effect on secondary haemostasis, whereas albumorm 5% had the least effect among colloids. Gelaspan 4% adversely affected the propagation phase of coagulation, maximal strength and elasticity of the coagulum, and the level of functional fibrinogen. All solutions adversely affected platelet function in primary haemostasis, with Plasma-Lyte showing the least effect.

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Introduction

Intensive and perioperative care frequently involve infusion therapy, aiming to replace circulating volume and maintain organ perfusion [1]. Currently, balanced solutions, predominantly crystalloids over colloids, are preferred [2]. Various crystalloid solutions with differing compositions are available, alongside colloids such as human albumin and semi-synthetically or synthetically produced solutions. The selection of a specific solution depends on the clinical situation and anticipated outcomes.

Crystalloids, containing electrolytes and/or low-molecular-weight sugars, do not increase plasma oncotic pressure after infusion and therefore pass relatively easily from the endovascular to the interstitial space. This results in approximately one-third to one-quarter of the administered crystalloid volume remaining in the

circulation. In contrast, colloids, due to their high molecular weight content (i.e., hydroxyethyl starch [HES], gelatine, dextran, albumin), increase oncotic pressure. Therefore, under conditions of intact endothelial glycocalyx, they pass extravascularly less readily, resulting in higher volume retention in the circulation and a more pronounced haemodynamic effect compared with crystalloids. However, when the glycocalyx is damaged (e.g., sepsis, trauma), permeability even for large molecules is increased. This increases the extravasation rate even for colloids and decreases their haemodynamic effect. It is important to highlight that the incorrect amount of solution administered may contribute to worsened morbidity and mortality [1, 2].

The composition of the solution can further impact mortality and morbidity. For example, saline solution, characterized by high concentrations of sodium chloride and a pH <6.0, has been associated with decreased renal flow and glomerular filtration [1, 2]. Moreover, solutions containing lactate as a buffering component are inappropriate, particularly in patients with higher serum lactate levels. Additionally, the use of HES, especially in patients with sepsis, has been associated with acute renal failure and the need for renal replacement therapy [1]. In contrast, the administration of albumin solution may confer benefits due to its role in maintaining oncotic plasma pressure, fluid dynamics at the microvascular level, glycocalyx stabilization, molecule transport, and antioxidant and immunomodulatory properties [1, 2].

Apart from variations in solution characteristics, differences in the impact on haemocoagulation status also distinguish them. This influence may stem from the dilution of the coagulation factors. Conversely, certain procoagulant effects of crystalloids and perhaps gelatine have been described [3]. Studies, investigating the effects of solutions on haemocoagulation, vary in the solutions used, involving 0.9% saline or other unbalanced solutions. Methodological differences also exist. Some studies assessed coagulation using standard tests, such as prothrombin time (PT) and activated partial thromboplastin time (aPTT), while others use viscoelastometric methods or investigate platelet function [4–15].

Sevcikova et al. performed an in vitro and in vivo study at our institution. Using rotational thromboelastometry, they found that a balanced crystalloid solution did not negatively affect haemocoagulation, whereas balanced colloid solutions could negatively affect the propagation phase of coagulation, coagulum strength, and functional fibrinogen levels [4, 5]. Among the colloids, however, she used the HES solution. In our department, we have not used this type of solution in recent years, and according to the European Medicines Agency recommendations, HES is not available in the market from 2022.

Therefore, this study aimed to evaluate the effects of currently available crystalloid and colloid solutions on

primary haemostasis using platelet function analyser (Innovance PFA-200, Siemens Healthineers, Germany). Given that haemocoagulation is more accurately described by the cellular model [16], we utilized the viscoelastometric method of rotational thromboelastometry (ROTEM, TEM International, Germany) to assess haemocoagulation in whole blood samples. This approach is more suitable for a comprehensive understanding of in vivo secondary haemostasis [17]. Unlike standard coagulation tests (PT, aPTT), rotational thromboelastometry provides a more detailed real-time analysis of coagulation processes [18, 19]. The objectives of this study were to determine the effect of Plasma-Lyte crystalloid solution, as well as colloid solutions containing gelatine (Gelaspan 4%) and human albumin (albumorm 5%) on haemocoagulation status, and to find a solution with minimal impact on haemostasis.

Methods

A total of 20 healthy subjects, with no history of coagulation disorders, no history of use of drugs affecting coagulation, and non-smokers, were included in the study. The subjects had a total of 14 mL of venous blood drawn by a single venipuncture using a 0.8 mm needle and a closed system (3.5 mL each into four VACUETTE tubes containing 3.2% sodium citrate). After sampling, each tube was gently inverted several times to ensure proper mixing of the blood with the anticoagulant. The samples were stored at room temperature (21–25°C) and processed within 60–120 min. During this period, the samples were carefully inverted at regular intervals until they were either analysed using PFA-200 or warmed to the operating temperature of 37°C for ROTEM analysis. For each test, 300 µL of citrated blood was used for ROTEM and 800 µL for PFA.

One blood sample served as a control, whereas the replacement solution (30 µL for ROTEM and 80 µL for PFA) was added to the remaining samples. The objective was to achieve a 10% dilution of the examined blood samples.

Replacement solutions were those commonly used in our department at present. These were balanced solutions of crystalloid (Plasma-Lyte, Baxter Czech, s. r. o.), 5% human albumin (albumorm 50 g/L, Octapharma, Belgium), and gelatine (Gelaspan 4%, B. Braun Melsungen AG, Germany).

According to the operating manual of the ROTEM delta machine [20], following tests were performed for each sample: EXTEM test (extrinsic coagulation pathway test, activated by calcium and recombinant tissue factor with phospholipids), FIBTEM (fibrinogen functional assay, activated by recombinant tissue factor with phospholipids and platelet blocker, cytochalasin D), and

Table 1. Effect of crystalloid and colloid solutions on ROTEM and PFA test parameters and their comparison with control sample

	Control	Plasma-Lyte	<i>p</i> value	albumorm 5%	<i>p</i> value	Gelaspan 4%	<i>p</i> value
EXTEM							
CT, s	72 (69–77)	68 (61–76)	0.005	71 (64–79)	0.082	71 (64–76)	0.021
CFT, s	82 (65–86)	79 (65–87)	0.980	86 (71–96)	0.001	91 (80–105)	<0.001
α , °	74 (73–77)	74 (72–77)	0.405	73 (71–75)	<0.001	72 (70–74)	<0.001
MCF, mm	63 (60–69)	64 (60–67)	0.143	62 (59–64)	<0.001	62 (59–66)	<0.001
LI60, %	89 (88–93)	90 (87–94)	0.151	92 (88–94)	0.199	93 (89–93)	0.051
INTEM							
CT, s	192 (155–243)	181 (171–211)	0.379	193 (159–227)	0.597	187 (163–222)	0.332
CFT, s	75 (59–90)	72 (60–84)	0.233	81 (68–94)	0.923	83 (71–96)	0.663
α , °	75 (72–78)	76 (73–78)	0.168	74 (72–77)	0.849	73 (71–76)	0.796
MCF, mm	60 (58–64)	61 (57–62)	0.697	58 (55–63)	0.038	58 (54–62)	<0.001
LI60, %	92 (87–94)	91 (88–93)	0.697	91 (88–94)	0.242	91 (88–94)	0.592
FIBTEM							
MCF, mm	14 (12–18)	13 (10–18)	0.017	13 (10–15)	0.001	12 (10–15)	0.009
PLTEM							
MCE	156 (136–204)	158 (140–186)	0.220	146 (131–163)	<0.001	145 (132–175)	<0.001
PFA							
Col/Epi-CT, s	187 (129–300)	189 (146–300)	0.459	202 (156–300)	0.030	186 (150–300)	0.131
Col/ADP-CT, s	89 (83–108)	106 (94–130)	0.003	127 (94–141)	<0.001	107 (98–130)	<0.001

Values are expressed as median with IQR. CFT, clot formation time; Col/ADP-CT, closure time in test with collagen/adenosine diphosphate; Col/Epi-CT, closure time in test with collagen/epinephrine; CT, clotting time; LI60, lysis index in 60 min; MCE, maximum clot elasticity; MCF, maximum clot firmness. Bold values are values with statistical significance of $p < 0.05$.

INTEM (intrinsic coagulation pathway assay, activated by the addition of calcium and ellagic acid with phospholipids). For the EXTEM and INTEM assays, the following parameters were monitored: CT (clotting time, corresponding to the initiation phase of coagulation), alpha angle and CFT (clot formation time, describing the propagation phase of coagulation), MCF (maximum clot firmness), and LI60 (lysis index at minute 60, describing the stability of the coagulum and fibrinolysis). The MCF parameter was monitored in the FIBTEM test, which evaluates the functional fibrinogen level [18, 20]. The MCE (maximum clot elasticity) parameters for the EXTEM and FIBTEM tests were calculated. Their difference (hereafter referred to as PLTEM-MCE) describes the elasticity of the coagulum, reflecting platelet function [21].

During the PFA examination, the CT parameter (closure time) was evaluated using Col/Epi (test containing collagen and epinephrine) and Col/ADP (test containing collagen and adenosine diphosphate). Both assays simulate adhesion and aggregation on a membrane containing an activator under conditions of high shear stress [18].

For statistical analysis, descriptive statistics methods were used, including determination of the mean, minimum and maximum values, median, and interquartile range of the observed parameters. Paired *T* tests were used to assess

differences between the samples, with p values <0.05 considered significant. Data analysis was performed using Statistica 14.0 software (TIBCO Software Inc.).

Results

We examined blood samples from 20 subjects (50% female, 50% male, median age 35 years, median BMI 24.8 kg.m⁻²). The results for each solution are listed in Table 1. Table 2 presents an inter-comparison of the solutions used. Comparison of the solutions with the control sample and inter-comparison in the ROTEM and PFA tests are presented in Figures 1 and 2.

Although albumorm had no statistically significant effect on the EXTEM-CT parameter compared to the control, the use of Plasma-Lyte and Gelaspan resulted in a significant reduction in this parameter. Thus, the partial procoagulant effects of both the solutions can be discussed. No statistically significant difference was observed when comparing Plasma-Lyte with Gelaspan.

In contrast, Gelaspan and albumorm demonstrated a hypocoagulant effect on the EXTEM-CFT and EXTEM-alpha parameters. Their use was associated with both a significant prolongation of the CFT parameter and a significant decrease in the alpha angle compared with the control. These parameters remained unaffected by Plasma-Lyte.

Table 2. Inter-comparison of crystalloid and colloid solutions in their effect on ROTEM and PFA test parameters

	Plasma-Lyte versus albumorm	Plasma-Lyte versus Gelaspan	albumorm versus Gelaspan
	<i>p</i> value	<i>p</i> value	<i>p</i> value
EXTEM			
CT	0.191	0.0764	0.220
CFT	<0.001	<0.001	0.018
α	0.809	0.653	0.202
MCF	<0.001	<0.001	0.202
LI60	>0.999	0.088	0.241
INTEM			
CT	0.565	0.764	0.321
CFT	0.059	0.005	0.119
α	0.068	0.009	0.179
MCF	0.028	<0.001	0.655
LI60	0.057	0.863	0.232
FIBTEM			
MCF	0.098	0.204	0.799
PLTEM			
MCE	<0.001	<0.001	0.256
PFA			
Col/Epi-CT	0.048	0.481	0.330
Col/ADP-CT	0.095	0.404	0.284

The numbers are *p* values. CFT, clot formation time; Col/ADP-CT, closure time in test with collagen/adenosine diphosphate; Col/Epi-CT, closure time in test with collagen/epinephrine; CT, clotting time; LI60, lysis index in 60 min; MCE, maximum clot elasticity; MCF, maximum clot firmness. Bold values are values with statistical significance of *p* < 0.05.

A significant difference was found in the EXTEM-CFT parameter between albumorm and Gelaspan, with the latter exhibiting the most pronounced effect on prolongation of this parameter. However, no significant differences were observed in the EXTEM-alpha parameters between these solutions.

Furthermore, albumorm and Gelaspan demonstrated a significant reduction in the maximum coagulum strength, as indicated by the EXTEM-MCF parameter, compared with the control. Thus, both solutions exhibited hypo-coagulant effects. Conversely, the use of Plasma-Lyte did not significantly affect the MCF parameter.

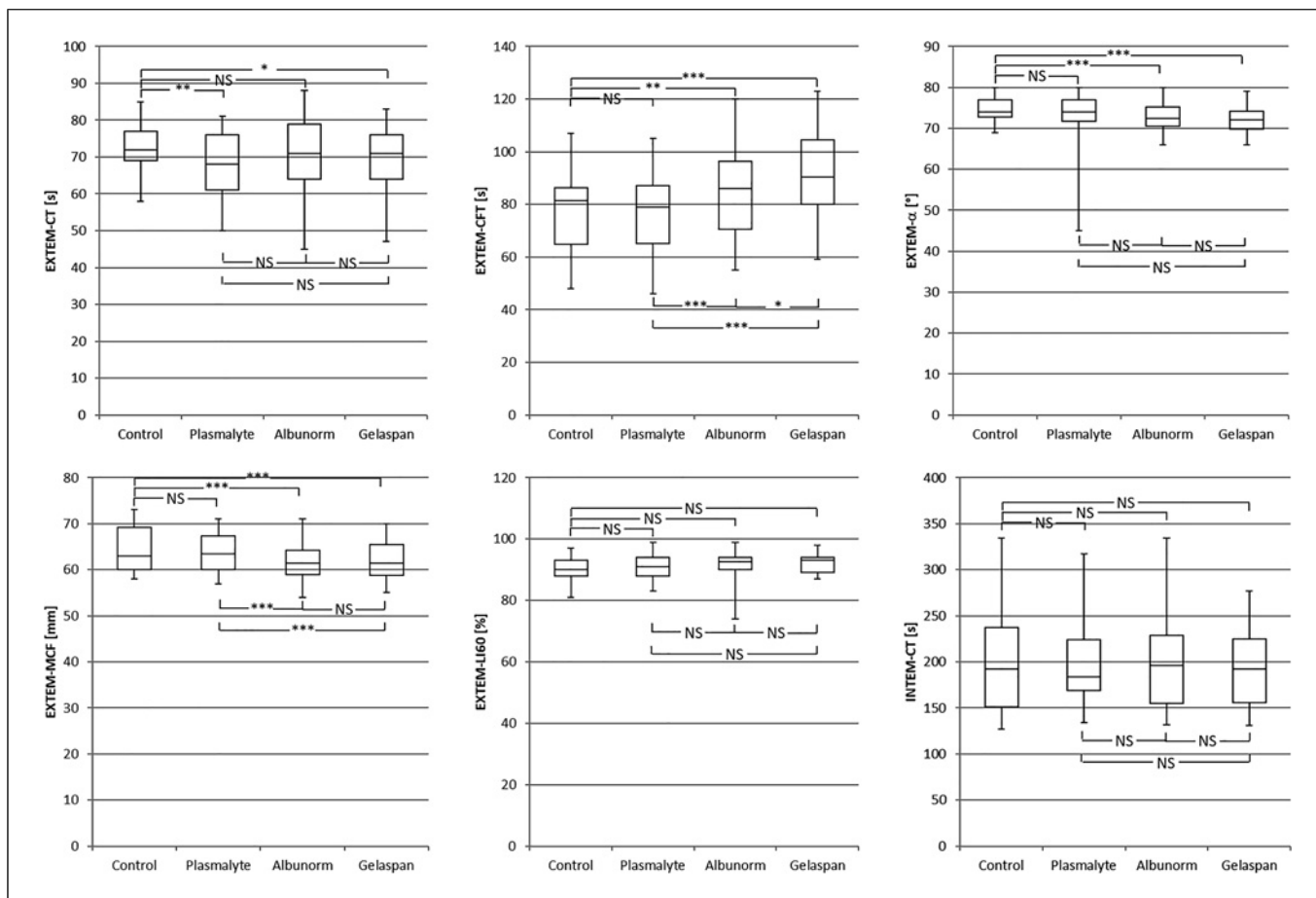
When comparing albumorm and Gelaspan, no significant differences were observed in the MCF parameters. None of the solutions had a significant effect on the EXTEM-LI60 parameter compared with the control. Similarly, no significant differences were observed when all the three solutions were compared.

The use of all solutions resulted in a significant decrease in the FIBTEM-MCF parameter compared with the control sample. The most notable difference was observed with the albumorm and Gelaspan, followed by Plasma-Lyte. However, no statistically significant differences were observed when the solutions were compared.

The use of Plasma-Lyte did not induce a change in the PLTEM-MCE parameter. However, the administration of both the colloid solutions led to a significant decrease in this parameter. No significant difference was observed between albumorm and Gelaspan.

None of the solutions produced a significant alteration in the INTEM-CT, INTEM-CFT, INTEM-alpha, or INTEM-LI60 parameters compared with the control. However, the administration of Gelaspan and albumorm resulted in a significant reduction in the INTEM-MCF parameter compared to the control sample. Conversely, Plasma-Lyte had no effect on the INTEM-MCF parameter.

Upon comparing the colloid solutions, no significant difference was observed in their influence on the MCF parameter. All solutions led to a significant prolongation of the CT parameter in the Col/ADP PFA test compared with the control. The least prolongation was observed in Plasma-Lyte. However, when comparing the solutions, no significant differences were observed. Only the administration of albumorm resulted in a significant prolongation of CT parameters in the Col/Epi PFA test. A significant difference was observed between albumorm



(Figure continued on next page.)

and Plasma-Lyte but not between alburnorm and Gelaspan.

Only the FIBTEM test exhibited a decrease in the MCF to just below the lower limit of normal for all solutions used. No changes outside the reference limits were observed in the other parameters. However, in the PFA tests, the use of all solutions resulted in a prolongation of the CT parameter, which in some cases exceeded the upper limit of normal and was unmeasurable.

Discussion

Infusion therapy exerts varying effects on the state of haemocoagulation, including a non-specific haemodilution effect as well as specific impacts of macromolecules present in colloid solutions [6, 22]. The effects of solutions on primary and secondary haemostasis have been investigated by several authors. However, individual studies differ in the solutions used (both unbalanced and balanced crystalloid and colloid solutions), the amount or degree of administered haemodilution, the methodology

used to assess haemostasis (including standard coagulation tests such as PT and aPTT, as well as TEG, ROTEM, PFA-100/200, and Multiplate), and the conditions under which the studies were conducted (whether in vitro or in vivo, involving human or animal subjects).

In summary of these studies, crystalloid or saline solutions, particularly at lower doses or dilution rates, have a less significant effect on haemostasis. Among colloids, albumin solutions exhibit the least impact on haemocoagulation at lower doses. They are followed by gelatine solutions and perhaps the most negative effects are attributed to HES, especially those with high molecular weight and high degree of substitution. In our study, we observed that all solutions adversely affected platelet function in primary haemostasis, with Plasma-Lyte having the least negative impact. No significant difference was found between the two colloids, alburnorm and Gelaspan. Plasma-Lyte also had the least negative effect on secondary haemostasis, causing only a reduction in functional fibrinogen levels. Additionally, both Plasma-Lyte and Gelaspan shortened the initiation phase, demonstrating a procoagulant effect. However, Gelaspan

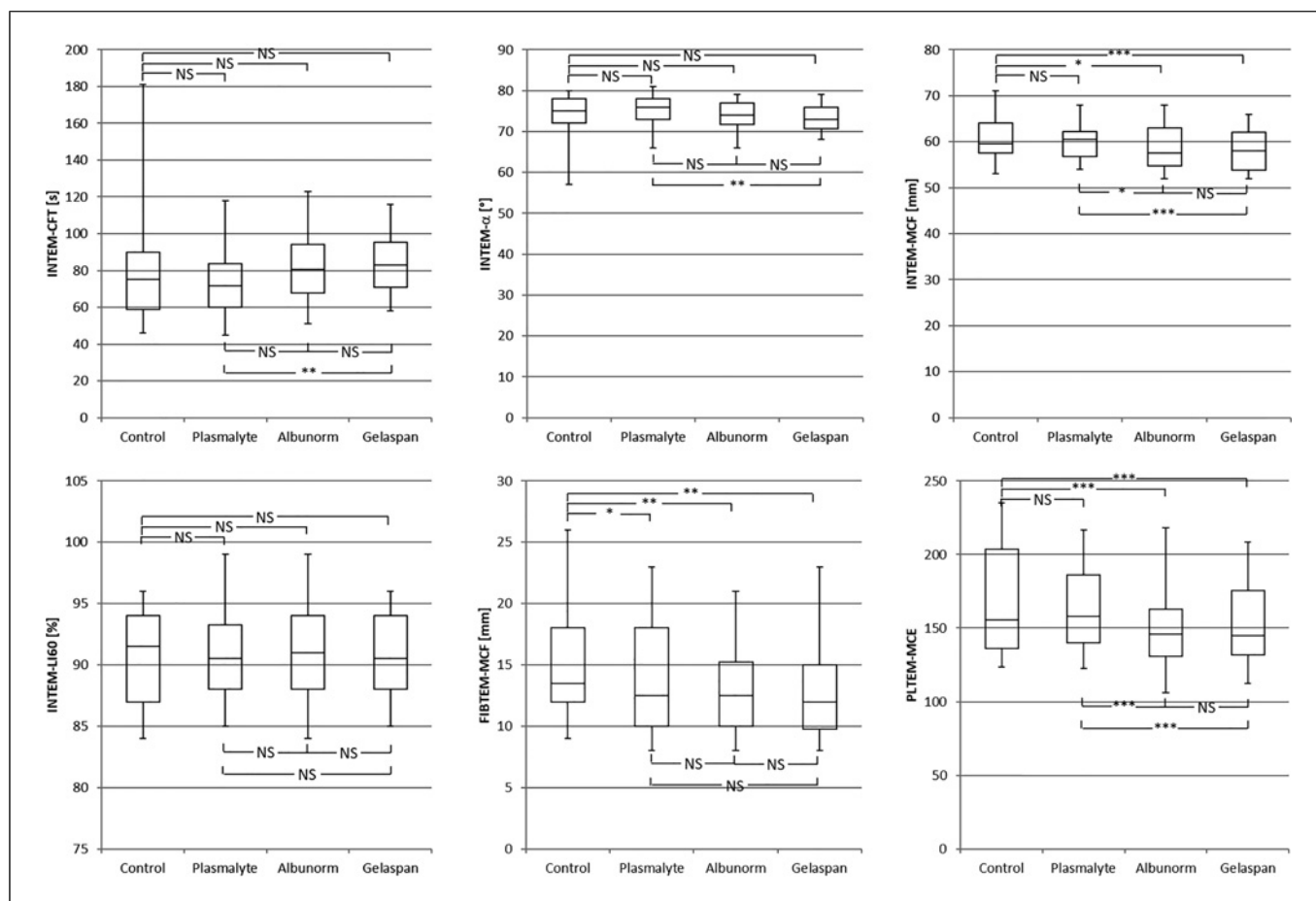


Fig. 1. Effect of crystalloid and colloid solutions on ROTEM test parameters and their comparison with the control sample and inter-comparison. CFT, clot formation time; CT, clotting time; EXTEM, extrinsic coagulation pathway test; FIBTEM, fibrinogen functional assay; INTEM, intrinsic coagulation pathway text; LI60,

lysis index in 60 min; MCE, maximum clot elasticity; MCF, maximum clot firmness; PLTEM-MCE, maximum clot elasticity in platelet function assay; ROTEM, rotational thromboelastometry. Statistical significance: NS, non-significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

and Albumin had a negative impact on the propagation phase of coagulation, maximal clot strength and elasticity, and functional fibrinogen levels. Gelaspan had a more adverse effect on the propagation phase compared to albumin.

Effect of Solutions on Primary Haemostasis

In our study, we observed a negative effect on primary haemostasis, as examined using the PFA-200 device, across all solutions used. This could be attributed to diminished platelet counts resulting from haemodilution as well as to the specific effects of the colloids. The potential influence of albumin on platelet reactivity has been indirectly suggested to occur through the binding of both pro-aggregating and anti-aggregating agents, such as prostaglandins [22]. Similarly, gelatine has been associated with adverse effects on platelet aggregation, von Willebrand factor, and factor VIII levels. Additionally, there are steric interactions between the macromolecules of infusion solutions and the platelet glycocalyx in which

the macromolecules are immobilized. This leads to a limitation of the access of other molecules to the platelet surface and other changes, e.g., in the ability of aggregation and adhesion or electrokinetic properties of the cells. In this way, the ability of the prothrombinase and tenase complex to bind to the surface of activated platelets may be affected. Steric effect has been particularly described for dextrans and HES and is dependent on their molecular weight [22–24].

The impact of HES on platelet function has been extensively investigated by Franz et al. [7]. They demonstrated that HES inhibits platelet function by reducing the expression of glycoprotein IIb/IIIa. The smallest inhibitory effect was observed for HES with a lower molecular weight (130 kDa) and a lower degree of substitution (0.38–0.45). In addition, they compared HES to saline. At a low dilution (induced by an infusion volume of $10 \text{ mL.kg}^{-1} \text{ b.w.}$), saline showed no significant effect on the prolongation of the CT parameter in the PFA Col/ADP test. However, at a 40% dilution (performed

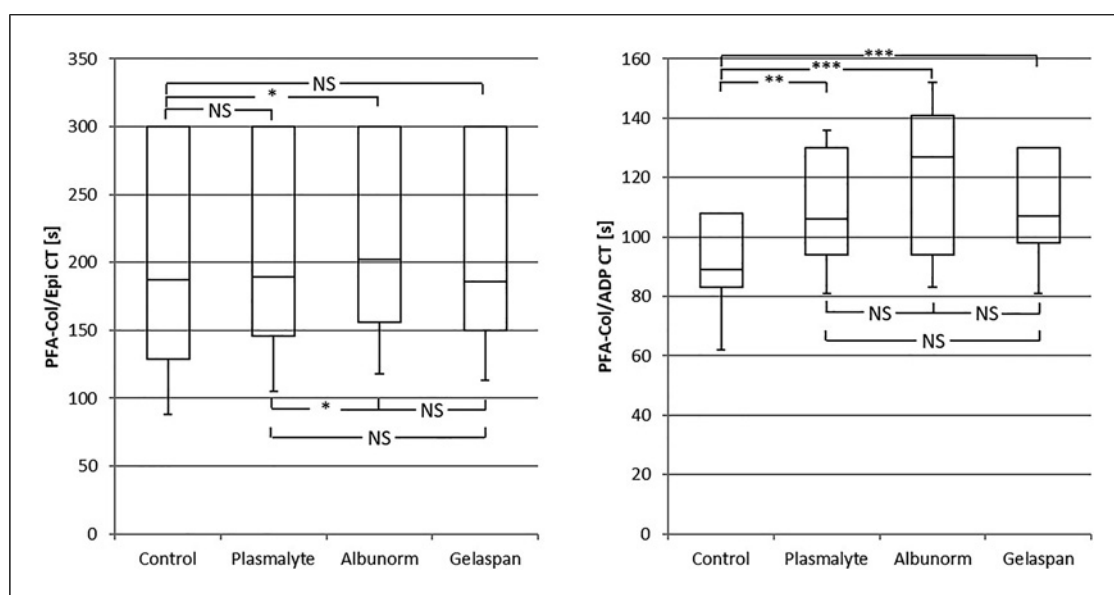


Fig. 2. Effect of crystalloid and colloid solutions on PFA test parameters and their comparison with the control sample and inter-comparison. Col/ADP-CT, closure time in test with collagen/adenosine diphosphate; Col/Epi-CT, closure time in test with collagen/epinephrine; PFA, platelet function analysis. Statistical significance: NS, non-significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

ex vivo), this parameter was significantly prolonged. Compared with HES, saline also resulted in the least prolongation of the CT parameter at this dilution. Similarly, in our study, the use of a crystalloid solution led to a prolongation of the CT parameter in the Col/ADP assay. An explanation for the observed prolongation, even at low dilution, may be attributed to differences in the methodology. While in our study dilution, dilution was conducted in vitro, in the aforementioned study, it was performed in vivo.

Boyd et al. [8] also observed that the crystalloid solution had the least significant effect on the CT parameter using the PFA Col/ADP test. Notably, they also used Plasma-Lyte solution similar to that used in our study. Succinylated gelatine (Gelofusine) had the most pronounced negative effect on the CT parameter, followed by HES 130/0.4 (Volulyte 6%).

Kind et al. [9] investigated the in vitro effect of 60% dilution using balanced and unbalanced gelatine and HES. They assessed platelet function utilizing the Multiplate device. Significant impairment of platelet function was observed in all solutions. Among these solutions, 4% balanced gelatine (Physiogel) had the least effect.

Li et al. [10] assessed the effects of 5% albumin and HES 130/0.4 on primary haemostasis in patients undergoing elective neurosurgery using the Multiplate device. No significant changes were observed when HES or a small volume of albumin (250 mL) was used. Only a negative effect was observed with a larger volume of albumin (500 mL) on test with adenosine diphosphate. It is important to highlight that this study administered

varying volumes of HES and albumin, and the patients also received different volumes of saline.

In a study by Sigurjonsson et al. [11], the albumin solution was also investigated. They evaluated the influence on primary haemostasis in gynaecological surgeries using the Multiplate device after administration of 5% albumin and 6% dextran. The use of either solution did not lead to pathological changes in the tests, and the solutions did not differ in the given parameters.

Effect of Solutions on Secondary Haemostasis

In our study, we used the ROTEM delta device to assess secondary haemostasis. During the initiation phase, we observed a procoagulant effect with Plasma-Lyte and, to a lesser extent, with Gelaspan, manifested by a reduction in the EXTEM-CT parameter. A similar effect, but for saline solution, was reported even at a 30% dilution by Shlimp et al. [12] in their in vitro study. No difference was observed for gelatine in this parameter. The CT parameters were prolonged when 5% albumin was administered, although the values remained within the reference range. In contrast, this threshold was exceeded when using HES 130/0.4. Zdolsek et al. [6] observed a mild procoagulant effect when using HES 130/0.42, 130/0.4, 200/0.5 and dextran 70, but they used CT parameter in NATEM test (native rotational thromboelastometry). Weiss et al. [13] did not observe this procoagulant effect in their study comparing saline and HES 130/0.4. However, in the case of saline, a significant prolongation of the EXTEM-CT parameter was noted up to 70% dilution, whereas with HES, this effect was

observed as early as 50% dilution. An explanation for the minimal or procoagulant effect of crystalloid administration could be attributed to both the rapid transfer of crystalloids to the extravascular environment and a more significant decrease in anticoagulant factors compared to procoagulant factors, albeit only up to a certain level of dilution. This disruption in the balance between coagulation and anticoagulation may subsequently lead to a procoagulant effect [14].

Moreover, we observed an adverse effect on the propagation phase as described by the CFT and alpha parameters. Gelaspan exhibited a more pronounced effect, whereas the effect was less significant for albumin. These alterations were statistically significant in the EXTEM test but not in the INTEM test. Conversely, Plasma-Lyte did not have a significant effect on this phase. Similar findings have been reported in other studies. While crystalloids or saline did not affect these parameters, the use of colloids resulted in a negative effect on the propagation phase, specifically the inhibition of the stable platelet-fibrin complex. Consistently, these studies documented a minor or negligible effect of albumin, contrasted with a more significant effect of gelatine and HES [12, 15].

Similar to our study, other studies have documented the impact of various solutions on the maximum coagulum strength in both the EXTEM and INTEM assays, depending on the degree of dilution. While the crystalloid solutions notably decreased the MCF parameter only at significant dilution, a reduction occurred at lower dilution levels with the use of colloids. Among these, albumin was associated with the smallest effect, whereas HES and gelatine exhibited a more pronounced impact [6, 12–15]. Given the modest dilution rate employed in our study, we did not observe a negative effect of the crystalloid on this parameter. Additionally, no significant difference was noted between the gelatine and albumin solutions.

The maximum coagulum firmness, described by the EXTEM-MCF parameter, is a result of fibrin polymerization and fibrin-stabilizing factor XIII activity in the presence of platelets. Thus, this parameter depends on the levels of functional fibrinogen, platelets, and FXIII activity. Consistent with our findings, other studies have reported a negative effect of solutions on fibrin polymerization, as evidenced by a reduction in the MCF parameter in the FIBTEM assay. This assay contains a platelet function blocker, enabling the exclusion of their influence on coagulum strength. Such effects were observed across all types of solutions and were contingent on the degree of haemodilution. Again, crystalloids demonstrated the least effect, followed by albumin and gelatine, with HES exhibiting the most significant impact [12–15].

The difference between the maximum elasticity of the coagulum in the EXTEM and FIBTEM tests was used to

evaluate the effect of platelets on the coagulum stability. Elasticity, unlike maximum firmness, better reflects the coagulum's resistance to forces during rotational movements in the device [21]. In our study, we observed a significant decrease in this parameter with both colloid solutions, while the crystalloid had no effect.

The solutions used in our study did not significantly affect fibrinolysis parameter (EXTEM-LI60). However, Egli et al. [14] in their study reported a negative effect of HES and gelatine on the coagulum stability. HES accelerates the conversion of fibrinogen to fibrin, leading to decreased fibrin stability and enhanced fibrinolysis. Similarly, gelatine adversely affects fibrin stability by interfering with fibrin polymerization upon integration into the nascent coagulum. Interestingly, saline even exhibited a decrease in parameters describing fibrinolysis with increasing dilution levels. Additionally, the albumin solution demonstrated a negative effect up to a dilution of 60%. Nonetheless, the fibrinolysis parameters remained within normal limits with all substitute solutions used.

A limitation of this study is its *in vitro* design, which restricts its direct translation to *in vivo* conditions. Also, we examined blood samples from healthy individuals. Consequently, our results may differ from those of studies conducted on patients. Moreover, patients receiving infusion therapy often have underlying conditions that can impact haemocoagulation status and influence the results of coagulation tests. Additionally, the study included a limited number of subjects and examined only one dilution level.

Considering the findings of this study and others, it can be concluded that the administration of a “standard” amount of balanced crystalloid has the least impact on coagulation parameters. In scenarios requiring the administration of a colloid solution, a 5% albumin solution has emerged as an ideal choice, exhibiting minimal effects on coagulation parameters compared to other colloids. It is also necessary to take into consideration the price of the individual solutions used. The production of human albumin solution is of course more expensive than in the case of synthetic colloids. In addition, due to regulations on the use of HES within the European Union, the costs associated with the higher consumption of albumin have increased. This raises the following questions for consideration – to use primarily balanced crystalloid solutions and to use colloid solutions only when acute hypovolemia cannot be adequately treated with crystalloids. If colloid is used, then albumin should be preferred in situations where the patient will benefit from simultaneous volume replacement and albumin substitution (hypoalbuminaemia, ascites, etc.) [25]. The suspension of registration for HES still applies in some EU countries. Publication of the results of studies investigating the safety and efficacy of 6% HES 130/0.4 (TETHYS and

PHOENICS study [26, 27]) is awaited. However, some authorities removed the suspension based on the delivered results of both studies. In view of the works mentioned in the discussion, their effect on coagulation is worse than that of albumin and gelatine but less than that of HES with higher molecular weight and higher degree of substitution. Therefore, the use of a specific solution should be individual for each patient and should be based on the patient's current need for the amount of volume replacement (respecting the maximum recommended doses), the urgency of its administration, the expected haemodynamic effect, the appropriate or beneficial composition of the solution, taking into account its adverse effects, including the haemocoagulation status.

Statement of Ethics

The study was approved by the Ethics Committee for Multi-centric Clinical Trials of Motol University Hospital, Prague, Czech Republic (Approval No. EK-1068/15). The entire research was performed in accordance with the Declaration of Helsinki. Informed consent was obtained from volunteers at the Department of Anaesthesiology and Intensive Care Medicine, Motol University Hospital, Prague, Czech Republic.

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

J.J. designed the study conception, collected the data, performed the statistical analyses, and wrote the manuscript. M.D. and T.V. reviewed the study conception and manuscript and contributed edits.

Data Availability Statement

All data analysed during this study are included in this article. Requests for the complete data set or further enquiries can be directed to the corresponding author (J.J.).

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