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Effects on coagulation of balanced (130/0.42) and non-balanced (130/0.4) hydroxyethyl starch or gelatin compared with balanced Ringer's solution: an *in vitro* study using two different viscoelastic coagulation tests ROTEM™ and SONOCLOT™†

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Key points

- Haemodilution with volume expanders has agent-specific effects on coagulation.
- The effects of haemodilution with balanced saline, hydroxyethyl starch, and gelatin on clot formation *in vitro* were investigated in normal blood.
- Using two independent assays of clot viscoelasticity (ROTEM™ and SONOCLOT™), both colloids had significantly greater negative effects on coagulation than balanced saline.

Background. Hydroxyethyl starch (HES) solutions compromise blood coagulation. Low molecular weight, low-substituted HES products, and electrolyte-balanced solutions might reduce this effect. We compared the effects of *in vitro* haemodilution on blood coagulation with a balanced 6% HES 130/0.42 solution (HES_{BAL}), a saline-based 6% HES 130/0.4 solution (HES_{SAL}), a balanced lactated Ringer's solution (RL) and a saline-based 4% gelatin solution (GEL).

Methods. Blood was obtained from 10 healthy male volunteers and diluted with the test solutions by 33% and 66%. Quality of clot formation was measured using two viscoelastic coagulation tests: SONOCLOT™ and activated rotation thromboelastometry ROTEM™.

Results. Of 16 parameters measured by the viscoelastic devices, we found three statistically significant differences compared with baseline for RL, but 11 for GEL, 10 for HES_{SAL}, and 11 for HES_{BAL} in the 33% haemodilution group ($P=0.01$). Comparing the different solutions, we observed a significant difference between crystalloids and colloids but none between GEL and HES. In the 66% dilution group, effects on blood coagulation were increased when compared with the 33% dilution group. We found no differences in coagulation impairment between balanced and non-balanced HES products and no differences in the detection of impaired blood coagulation due to haemodilution between the two viscoelastic coagulation tests.

Conclusions. Both ROTEM™ and SONOCLOT™ are sensitive tests for the detection of impaired blood coagulation due to haemodilution. There are fewer effects on blood coagulation using crystalloids compared with colloids. The effects of GEL and HES are similar. There is no difference between balanced HES 130/0.42 and non-balanced HES 130/0.4.

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During major surgery, administration of synthetic plasma expanders protects the patient from adverse effects of allogeneic blood transfusions such as transmission of infectious diseases, increases in mortality, major morbidity, transfusion-related acute lung injury, nosocomial infections, increased costs, and immunological effects, including tumour growth promotion.[1–6] Colloids and crystalloids influence blood coagulation in different ways. With increasing use, an additional influence on coagulation by the artificial plasma expander itself might become clinically relevant beyond the effect that coagulation factors and platelets are diluted. *In vivo* and *in vitro*, crystalloids show less effect on blood coagulation than colloids, gelatin shows less effect than hydroxyethyl starch (HES), and dextrans show the greatest negative coagulation impact of the colloids.[7–11] Third-generation 6% HES 130/0.4 with a lower molecular weight and a lower molar substitution shows a significantly reduced negative impact on blood coagulation *in vivo* and *in vitro* compared with early HES products.[12–14] In addition, some studies suggest less coagulation impairment by HES

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preparation in a balanced solution with physiological, plasma-adapted electrolyte concentrations.[15 16]

The purpose of this study was to assess the effects of *in vitro* haemodilution on blood coagulation of commonly used volume expanders: 6% HES in a balanced 130/0.42 and non-balanced 130/0.4 solution compared with 4% gelatin solution, and a balanced Ringer's lactate-crystalloid solution. Apart from standard coagulation variables, we assessed the quality of clot formation using two different viscoelastic devices, SONOCLOT™ and ROTEM™. In addition, we evaluated and compared these two point-of-care devices.

Methods

After approval by the institutional ethics committee (Kantonale Ethik-Kommission des Kantons Luzern, protocol number 686) and written informed consent, 10 healthy volunteers (28–52 yr of age) were enrolled. Inclusion criteria for the study were male sex, no history of chronic or acute diseases, no intake of any medication, in particular acetylsalicylic acid or non-steroidal anti-inflammatory agents within 2 weeks before the study, no alcohol or drug abuse, or smoking. Blood samples were obtained from an antecubital vein using an 18 G venous cannula (Vasofix™, B. Braun, Melsungen, Germany). To exclude blood coagulation activation due to venous stasis, vein puncture, or blood withdrawal, we discarded 5 ml of native blood (whole blood) before 2 ml of native blood for SONOCLOT™ analysis and 4 ml of citrated blood for thromboelastometry were taken for each measurement. The different plasma expander solutions were immediately diluted using polystyrene tubes and the coagulation measurements started within 2 min after having obtained the blood sample. After measurement of an undiluted sample, the blood was diluted 33% and 66% by volume (as in former investigations with similar interrogations),[8 9 12 16] using as test solutions: an electrolyte plasma-adapted, 'balanced' Ringer's solution (containing Na^+ 140 mM, Cl^- 127 mM, K^+ 4.0 mM, Ca^{2+} 2.5 mM, Mg^{2+} 1.0 mM, acetate 24.0 mM, and malate 5.0 mM, *Ringerfundin*™, B. Braun), a conventional saline-based 4% gelatin solution (containing Na^+ 154 mM and Cl^- 120 mM, *Gel 4%*™, B. Braun), a conventional, non-balanced 6% HES 130/0.4/9:1 solution (6%=concentration/130 kDa=mean molecular weight/0.4=molar substitution ratio, the average number of hydroxyethyl groups per unit of glucose/9:1=C2:C6 ratio, the distribution of the hydroxyethyl units between the C2 and C6 positions of the glucose unit) containing Na^+ 154 mM and Cl^- 154 mM, *Voluven*™, Fresenius Kabi, Bad Homburg, Germany), and a balanced 6% HES 130/0.42/6:1 solution, based on potato as the biological source (containing Na^+ 140 mM, Cl^- 118 mM, K^+ 4.0 mM, Ca^{2+} 2.5 mM, Mg^{2+} 1.0 mM, acetate 24.0 mM, and malate 5.0 mM, *Tetraspan 6%*™, B. Braun).

Routine laboratory tests were done with the automated analysing systems at the central laboratory. All blood samples for routine laboratory tests were obtained at once, whereas native blood samples for SONOCLOT™ and citrated blood samples for ROTEM™ had to be obtained with an 18 G venous cannula shortly before processing. The dilutions were prepared as mentioned above and the following parameters were analysed: haematocrit, haemoglobin concentration, platelet count, fibrinogen, activated partial thromboplastin time (aPTT) and prothrombin time (PT) with international normalized ratio.

To rule out a platelet defect, we used the Platelet Function Analyzer PFA-100™ (Dade/Behring, Marburg, Germany) and investigated the initial steps of platelet-mediated blood coagulation using ADP and epinephrine stimulation.[17]

Analysis of clot formation was performed with two whole-blood coagulation tests: SONOCLOT™ (Sonoclot II Coagulation and Platelet Function Analyzer, Sienco Co., Morrison, CO, USA) and activated rotation thromboelastometry (ROTEM™, Pentapharm, Munich, Germany). The principle of these tests is the analysis and graphical presentation of changes in viscoelastic properties of clot formation and retraction and subsequent fibrinolysis. The tests are sensitive to impairment of clot formation involving coagulation factors and inhibitors, platelets, and fibrinogen. Technical details of the two coagulation analysers and their parameters have been described previously.[18]

SONOCLOT™

To accelerate the coagulation process, the cuvette contains 5 mg of Celite to activate factor XII at the beginning of the intrinsic pathway. As determined in other SONOCLOT™-based trials,[8 19] we evaluated the following parameters: activated clotting time (ACT), clot rate (CR), peak amplitude (PA), and time to peak (TTP). ACT is the time from test start until the beginning of fibrin formation and corresponds to the conventionally measured ACT. Fibrin formation (Gel phase) starts when the viscosity of the sample begins to increase by fibrin formation from fibrinogen and is characterized by the primary slope of the curve (CR). After completion of clot formation, PA reflects the fibrinogen concentration. TTP characterizes clot retraction and describes the speed and quality of fibrin formation[8 18–20] (Fig. 1).

ROTEM™

Citrated-anticoagulated blood (0.3 ml) was re-calcified with 20 μl calcium chloride. Activation of coagulation was performed as follows: extrinsic thromboelastometry (Extem) with 20 μl of tissue thromboplastin, intrinsic thromboelastometry (Intem) with 20 μl of the surface activator partial thromboplastin, and native thromboelastometry (Natem) without activator, only with re-calcification. We determined the following parameters: onset of coagulation [coagulation time (CT)], kinetics of clot formation [clot formation time (CFT): the time elapsed between 2 and 20 mm clot firmness], the Alpha angle (angle of a tangent to the clotting curve through the 2 mm point), and maximum clot firmness (MCF). All measurements were performed within 2 min after blood withdrawal at 37°C using reagents from the manufacturer (Fig. 2).

Statistical analysis

Statistical analysis was done using the commercially available software package Statistica Version 7.0 (StatSoft Inc., Tulsa, OK, USA) and StatExact 7.0 (Cytel Software

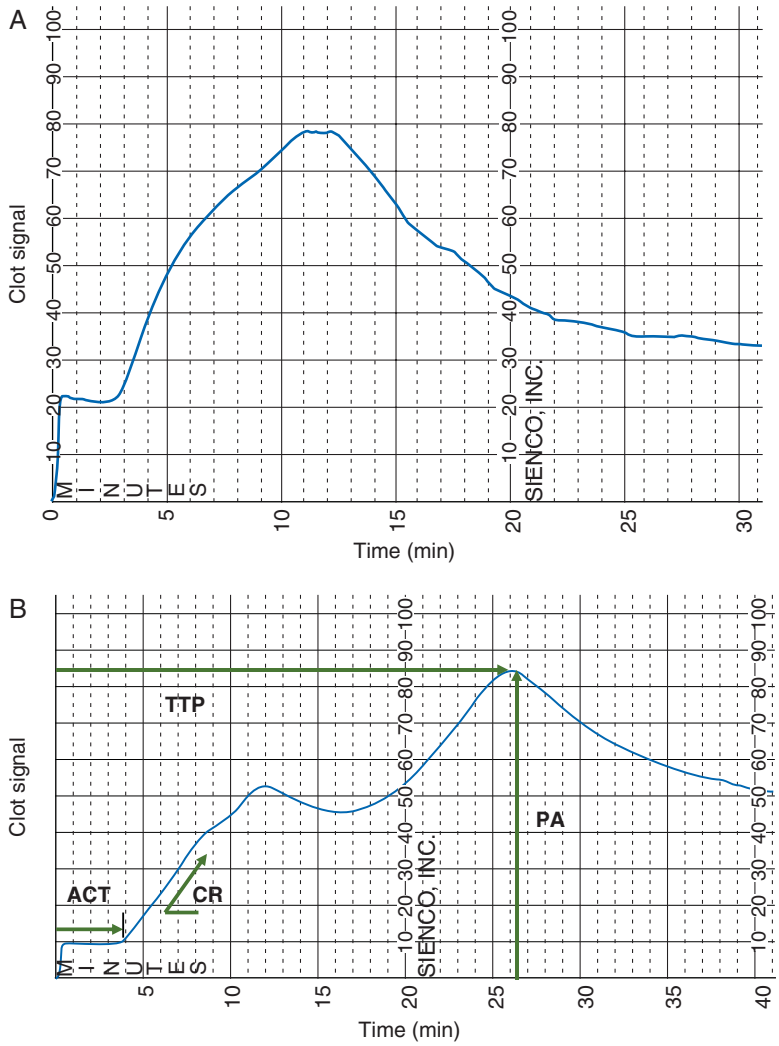

Fig 1 (A) Normal SONOCLOT™ curve. (B) SONOCLOT™ curve after 66% haemodilution with balanced-electrolyte lactated Ringer's solution (RL). ACT, activated clotting time; CR, clot rate; PA, peak amplitude; TTP, time to peak.

Corporation, Cambridge, MA, USA). Non-parametric analysis was performed with one-way analysis of variance (ANOVA) equivalent test procedure with multiple testing and pair characteristic of the data; *post hoc* comparison with the Bonferroni correction was applied to adjust the level of significance. Subsequently, the Fisher–Freeman–Halton test was applied to analyse the categorical data of inhomogeneous distribution of the coagulation tests by haemodilution.

Because of the obvious effect of the two dilutional steps, the statistical differences are not shown. Of further interest are the differences between the solutions at their various levels of dilution. Data are mean (SD). A P-value <0.05 was considered statistically significant.

Results

Ten volunteers with a median body weight (range) of 78 (70–86) kg and body height of 181 (175–187) cm were enrolled. All baseline values including PT, aPTT, fibrinogen, haematocrit, haemoglobin concentration, and platelet count were within the normal range. Platelet dysfunction was excluded by normal PFA measurements (ADP- and epinephrine-closure time) (Table 1). After dilution, fibrinogen, haematocrit, haemoglobin concentration, and platelet count decreased, and PT and aPTT increased, indicating compromised blood coagulation (data not shown). Furthermore, all baseline variables measured by SONOCLOT™ and ROTEM™ were within normal limits as defined by the manufacturers.

SONOCLOT™

After haemodilution, ACT and TTP were prolonged and CR and PA decreased, representing the negative influence of dilution on haemostasis.

33% haemodilution. Compared with baseline, ACT showed a significant prolongation for all agents. Additionally, CR decreased and TTP increased for all colloids (Table 2).

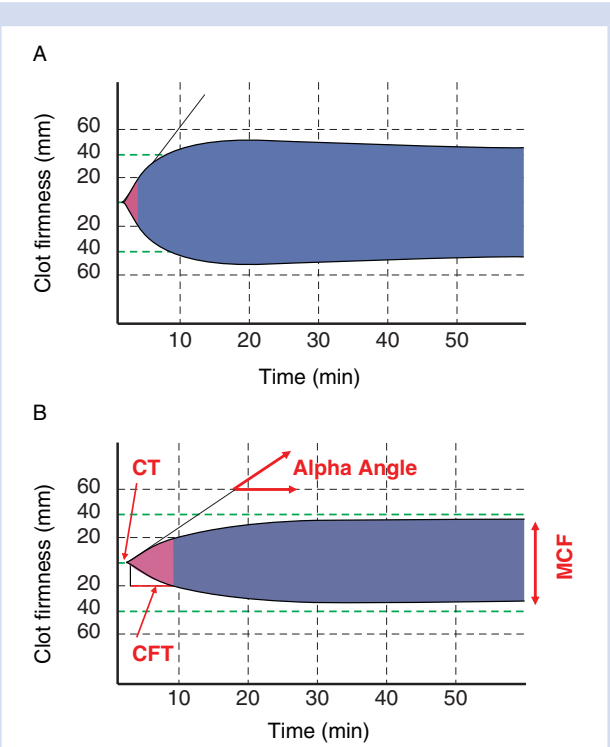

Fig 2 (A) Normal Extem ROTEM™ curve. (B) Extem ROTEM™ curve after 66% haemodilution with balanced-electrolyte lactated Ringer's solution (RL). CT, coagulation time; CFT, clotting formation time; MCF, maximum clot firmness.

Table 1 Baseline coagulation and platelet variables of the 10 volunteers. ADP, adenosine diphosphate; aPTT, activated partial thromboplastin time; Hb, haemoglobin; Hct, haematocrit; INR, international normalized ratio; PFA, platelet function analyzer; PT, prothrombin time; SD, standard deviation

	Mean (SD)	Range
Platelet count (10^3 μl^{-1})	220 (41)	175–289
Hb (g l^{-1})	153 (10)	140–172
Hct (%)	43 (3)	40–49
Fibrinogen (g l^{-1})	2.5 (0.5)	1.8–3.4
PT (%)	98 (10)	85–118
INR	1.03 (0.05)	0.95–1.10
aPTT (s)	30 (2)	28–32
PFA 100-closure time (s)		
ADP	88 (12)	65–110
Epinephrine	141 (30)	106–205

66% haemodilution. ACT increased and CR decreased significantly compared with baseline regarding all agents. TTP increased significantly for all colloids compared with baseline (Table 3).

ROTEM™

After haemodilution, CT was prolonged, Alpha angle and MCF decreased, and CFT increased.

Table 2 Statistical differences between agents at 33% haemodilution compared with undiluted baseline. GEL, saline-based gelatin solution; RL, balanced-electrolyte lactated Ringer's solution; HES_{BAL}, balanced-electrolyte HES solution; HES_{SAL}, saline-based HES solution; ACT, activated clotting time; CFT, clot formation time; CR, clot rate; CT, coagulation time; MCF, maximum clot firmness; ns, not significant; PA, Peak amplitude; *P*, statistical difference of sum; sum, sum of statistical different coagulation tests

33%	ACT	CR	TTP	PA	Extem-CT	Extem-CFT	Extem-Alpha	Extem-MCF	Intem-CT	Intem-CFT	Intem-Alpha	Intem-MCF	Natem-CT	Natem-CFT	Natem-Alpha	Natem-MCF	Sum	*P*-value
RL	0.03	NS	NS	NS	NS	NS	NS	<0.01	NS	NS	NS	<0.01	NS	NS	NS	NS	3	0.01
GEL	<0.01	<0.01	0.02	NS	NS	0.01	<0.01	<0.01	NS	0.02	<0.01	<0.01	0.03	NS	NS	<0.01	11	NS
HES_{SAL}	<0.01	<0.01	<0.01	NS	NS	<0.01	<0.01	<0.01	NS	<0.01	<0.01	<0.01	NS	NS	NS	<0.01	10	NS
HES_{BAL}	0.01	<0.01	<0.01	NS	0.03	<0.01	<0.01	<0.01	NS	<0.01	<0.01	<0.01	NS	NS	NS	<0.01	11	NS

Table 3 Statistical differences between agents at 66% haemodilution compared with undiluted baseline. GEL, saline-based gelatin solution; RL, balanced-electrolyte lactated Ringer's solution; HES_{BAL}, balanced-electrolyte HES solution; HES_{SAL}, saline-based HES solution; ACT, activated clotting time; CFT, clot formation time; CR, clot rate; CT, coagulation time; MCF, maximum clot firmness; NS, not significant; PA, Peak amplitude; *P*, statistical difference of sum; sum, sum of statistical different coagulation tests

66%	ACT	CR	TTP	PA	Extem-CT	Extem-CFT	Extem-Alpha	Extem-MCF	Intem-CT	Intem-CFT	Intem-Alpha	Intem-MCF	Natem-CT	Natem-CFT	Natem-Alpha	Natem-MCF	Sum	*P*-value
RL	<0.01	<0.01	NS	NS	NS	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	NS	<0.01	<0.01	<0.01	12	NS
GEL	<0.01	<0.01	<0.01	NS	NS	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	NS	<0.01	<0.01	<0.01	13	NS
HES_{SAL}	<0.01	<0.01	0.01	NS	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	NS	<0.01	<0.01	<0.01	14	NS
HES_{BAL}	<0.01	<0.01	0.01	NS	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.03	<0.01	<0.01	<0.01	15	NS

33% haemodilution. Extem-CT was significantly prolonged compared with baseline only for HES_{BAL}. We found a significant Extem-CFT increase and Extem-Alpha decrease between baseline and after dilution by colloids. Extem-MCF decreased significantly for all agents. The colloids showed a significant Intem-CFT increase and Intem-Alpha decrease compared with baseline. All agents showed a significant decrease in Intem-MCF compared with baseline. Natem-CT was only significantly prolonged between GEL and baseline. Natem-MCF showed a significant decrease to baseline for the colloids (Table 2).

66% haemodilution. In Extem-CT, we found a significant prolongation between baseline and HES products. All agents significantly increased Extem-CFT and decreased Extem-Alpha and Extem-MCF compared with baseline. All agents were significantly different from baseline in the Intem measurements (increase in CT and CFT and decrease in Alpha and MCF). In Natem-CT, a significant prolongation was only observed between baseline and HES_{BAL}. Natem-CFT significantly increased and Natem-Alpha and Natem-MCF decreased between baseline and all agents (Table 3).

Summarized results of SONOCLOT™ and ROTEM™

We compared the number of significant differences in SONOCLOT™- and ROTEM™-parameters (a total of 16 values). We found three statistical significant differences compared with baseline values for RL, 11 for GEL, 10 for HES_{SAL}, and 11 for HES_{BAL} (Table 2). The Fisher–Freeman–Halton test showed a significant difference between the agents listed in Table 2 ($P<0.01$). There were no significant differences between the agents in the Freeman–Halton test for the 66% haemodilution group (Table 3). The intergroup analysis showed a pronounced reduction of blood coagulation by the use of colloids compared with crystalloids in both dilution groups (33% and 66%) (Tables A1 and A2).

SONOCLOT™ *vs* ROTEM™ and *vs* haematological parameters

Baseline haematological laboratory values were correlated with the readout variables of SONOCLOT™ (ACT, CR, PA, and TTP) and ROTEM™ (CT, CFT, and MCF with the activation paths Extem, Intem, and Natem). A statistically significant correlation was found between fibrinogen level and Intem-MCF ($r=0.79$) and between platelet count and Natem-MCF ($r=0.73$). No correlation could be found between ACT and Extem-, Intem-, or Natem-CT. CR correlated significantly with Extem-CT ($r=0.66$) and Intem-CFT ($r=0.74$). TTP also correlated significantly with Intem-CT ($r=0.66$) and Intem-MCF ($r=0.83$). The MCF values as measured with the Extem and Natem activation paths of ROTEM™ also correlated but not with Intem-MCF value. CF correlated significantly with TTP ($r=0.64$) but only with Intem-CFT ($r=0.74$). When using a cluster analysis, ACT can most suitably be linked to Extem-CFT and Intem-CFT.

Discussion

We found a pronounced inhibitory effect of colloids compared with crystalloids on blood coagulation after 33% haemodilution *in vitro*. There were no differences in the effects on blood coagulation between the gelatin solution and both HES products, or between the balanced- and the saline-based HES solutions. There was no difference between the colloids in the 66% haemodilution groups. The intergroup comparisons confirmed the pronounced reduction of blood coagulation by the use of colloids compared with crystalloids in both dilution groups.

Intravascular volume expanders inhibit blood coagulation concentration dependently. A mild-to-moderate haemodilution with crystalloids can accelerate blood coagulation *in vitro*[8 9] and *in vivo*.[21–23] An imbalance between decreased antithrombin III activity and thrombin generation can partly explain this effect.[21] In contrast, the use of colloids for haemodilution leads to compromised blood coagulation as shown in most studies. Gelatin solutions might influence the weight and reticular network of fibrin strands and platelet function by ristocetin and polybrene with decreased von Willebrand factor.[24 25] However, other studies have shown only a moderate effect[9 26] or indicate that blood coagulation might even be accelerated.[8 11] HES compromises blood coagulation significantly as *in vitro*[8 9 12 13] and *in vivo* studies have shown.[11 27 28] There are several mechanisms which influence blood coagulation, for example, an acquired von Willebrand syndrome and decreasing effect on factor VIII and decreased expression and activation of platelet surface GPIIb–IIIa receptor with impaired platelet adhesion and aggregation.[13 14] A further mechanism appears to be the impaired polymerization of fibrin monomers by HES macromolecules.[11 13] In addition to the quantity of the administered HES, the chemical properties of the molecules play an important role in blood coagulation. A high molecular weight seems to be of minor importance compared with a high molar substitution.[29] A reduction of the C2/C6 ratio results in faster HES elimination without influence on blood coagulation.[30] Low molecular weight, low-substituted 6% HES 130/0.4 possesses a lower molecular weight and a lower molar substitution, and many trials have observed less negative impact on blood coagulation compared with early HES products.[12–14 31 32] When HES is prepared in a balanced solution with physiological electrolyte concentrations, impaired coagulation is reduced.[15 16 33]

We did not find evidence for hypercoagulation during haemodilution with crystalloids. All measured coagulation parameters remained unchanged or showed a tendency towards a hypocoagulable condition. Although balanced Ringer's solution should theoretically compromise coagulation only to a low extent due to the additional calcium ions, we could not confirm this hypothesis. The ROTEM™ device is not suitable for such examinations as the citrated blood samples are re-calcified resulting in a surplus of calcium ions in the sample. In contrast to the ROTEM™ device, SONOCLOT™ only uses native blood samples and does not need additional calcium. But, there is no indication of a minor influence of balanced volume expanders on haemodilution compared with saline-based volume expanders in the SONOCLOT™ measurements either.

Furthermore, we did not observe significant differences between balanced and non-balanced HES products. Balanced solutions do not possess high and unphysiological concentrations of sodium and chloride (each 154 mM) as saline-based volume expanders do. Therefore, the risk of developing a hyperchloraemic acidosis after administration of large amounts of such solutions is decreased.[34 35] Boldt and colleagues[36] reported no differences between balanced 6% HES 130/0.42 together with a balanced crystalloid solution and non-balanced 6% HES 130/0.42 together with an unbalanced saline solution in an *in vivo* study. They found no differences between the two groups regarding Intem-CT, Intem-CFT, and Intem-MCF at baseline and at postoperative day 1. In a follow-up *in vitro* study, Boldt and colleagues described a more negative effect on coagulation using non-balanced 6% HES 130/0.4 compared with balanced 6% HES 130/0.42. In the 50% dilution group, they found more pronounced changes in Intem-MCF, Intem-CFT, Extem-CT, Extem-CFT, and Extem-MCF when using the non-balanced solution compared with the balanced solution, whereas in the 30% dilution group, changes were found in Extem-CFT and Extem-CT.[16] As mentioned above, ROTEM™ is not a suitable device for examining the difference between a balanced and a non-balanced volume expander by an *in vitro* test. In contrast, in our study, we performed an additional viscoelastic coagulation assessment and evaluated a total of 16 test parameters of both the ROTEM™ and the SONOCLOT™ devices.

For both viscoelastic devices, a high sensitivity is required to identify a haemodilution coagulopathy. ACT, CR, and TTP seem to be sensitive parameters for detecting haemodilution coagulopathies of the SONOCLOT™ analysis, whereas ACT is the most sensitive of all parameters (providing statistical significant results for all tested agents in our study). Regarding the ROTEM™ analysis, CFT, Alpha, and MCF are sensitive parameters with MCF as the most sensitive one. Extem and Intem measurements are more or less equally sensitive, whereas Natem obviously possesses a lower sensitivity. Natem represents the rotation thromboelastography without activators only with re-calcification. Therefore, it should be very similar to conventional TEG measurements. Although fibrin-polymerization is relevant in dilutional coagulopathy and Fibtem is a sensitive test, we decided not to include the Fibtem results in our statistical analysis. Fibtem has only one important parameter (MCF) which showed no difference to the Extem-, Intem-, or Natem-MCF results and had no additional value for our study since we statistically compared significances of the test measurements with the Fisher–Freeman–Halton test—so Fibtem would be the 17th test measurement and would not change our results. Furthermore, in 66% haemodilution with colloids, the Fibtem was frequently technically not possible.

Interestingly, the ACT values of SONOCLOT™ and ROTEM™ cannot be compared with each other. Our observation of a

complex pattern of correlations between the different variables of the devices shows that the two different viscoelastic methods introduce different ACT analyses. ROTEM™-CT describes the time between the beginning of pin rotation until the pin begins to be impaired after fibrin–platelet bonding has linked cup and pin.[18] The vertical oscillation in the sample of the SONOCLOT™ device is sensitive to viscosity changes during initiation of coagulation. There are further different activators: Celite for SONOCLOT™, tissue thromboplastin for Extem, partial thromboplastin for Intem, or no activator for Natem. The two different procedures and the different activators might explain the lack of statistic correlation between the two tests.

A limitation of this *in vitro* study is the absence of physiological reaction during haemodilution including recruitment of additional resources of the coagulation system, changes in plasma ions and acid–base balance, tissue damage, and endothelial injury with their pro-coagulation properties, or interaction between the volume expander and the endothelial system. Further, the different colloid solutions have different half times *in vivo* (HES>GEL), so that *in vivo* testing might generate different results compared with *in vitro* testing. *In vivo* studies would be necessary to confirm our observations in the clinical setting. Another limitating factor in our study is the difference in the two HES products which are used in our institution. They are from two different companies and possess different molar substitution ratios (0.42 for the balanced HES solution and 0.4 for the saline-based HES solution) and a different C2:C6 ratio (6:1 for the balanced HES solution and 9:1 for the saline-based HES solution).

In conclusion, haemodilution negatively affected blood coagulation *in vitro* as measured by the viscoelastic devices SONOCLOT™ and ROTEM™, with less pronounced changes when a balanced-electrolyte lactated Ringer's solution is used when compared with colloidal agents. Saline-based gelatin solution did not show greater influence on haemostasis than HES 130/0.4 (only a tendency in intergroup analysis). There is no difference between the balanced HES solution and the saline-based HES solution. Both viscoelastic devices, ROTEM™ and SONOCLOT™, are sensitive to changes in blood coagulation during haemodilution, with MCF being the most sensitive parameter in ROTEM™ analysis and ACT in SONOCLOT™ analysis.

Conflict of interest

In the past 5 yr, D.R.S. has received honoraria or travel support for consulting or lecturing from the following companies: Abbott AG, Baar, Switzerland; Alliance Pharmaceutical Corp., San Diego, CA, USA; AstraZeneca AG, Zug, Switzerland; Bayer (Schweiz) AG, Zürich, Switzerland; B. Braun Melsungen AG, Melsungen, Germany; CSL Behring GmbH, Hattersheim am Main, Germany; Fresenius SE, Bad Homburg v.d.H., Germany; Galenica AG, Bern, Switzerland (including Vifor SA, Villars-sur-Glâne, Switzerland); GlaxoSmithKline GmbH & Co. KG, Hamburg, Germany; Janssen-Cilag AG, Baar, Switzerland; Novo Nordisk A/S, Bagsvärd, Denmark, Octapharma AG, Lachen, Switzerland; Organon AG, Pfäffikon/SZ, Switzerland; and Roche Pharma (Schweiz) AG, Reinach, Switzerland.

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Institute of Anesthesiology and Intensive Care, Kantonsspital, Lucerne, Switzerland.

Appendix

Table A1 Intergroup comparisons of 33% haemodilution with different volume expanders ($P<0.05$ was considered statistically significant). GEL, saline-based gelatin solution; RL, balanced-electrolyte lactated Ringer's solution; HES_{BAL}, balanced-electrolyte HES solution; HES_{SAL}, saline-based HES solution; ACT, activated clotting time; CFT, clot formation time; CR, clot rate; CT, coagulation time; MCF, maximum clot firmness; NS, not significant; PA, peak amplitude

		RL	GEL	HES_{SAL}	HES_{BAL}
ACT	RL		NS	NS	NS
	GEL	NS		NS	NS
	HES_{SAL}	NS	NS		NS
	HES_{BAL}	NS	NS	NS	
CR	RL		0.01	<0.01	<0.01
	GEL	0.01		NS	NS
	HES_{SAL}	<0.01	NS		NS
	HES_{BAL}	<0.01	NS	NS	
TTP	RL		NS	<0.01	<0.01
	GEL	NS		NS	NS
	HES_{SAL}	<0.01	NS		NS
	HES_{BAL}	<0.01	NS	NS	
PA	RL		NS	NS	NS
	GEL	NS		NS	NS
	HES_{SAL}	NS	NS		NS
	HES_{BAL}	NS	NS	NS	
Extem-CT	RL		NS	NS	0.01
	GEL	NS		NS	0.01
	HES_{SAL}	NS	NS		NS
	HES_{BAL}	0.01	0.01	NS	
Extem-CFT	RL		NS	NS	0.01
	GEL	NS		NS	NS
	HES_{SAL}	NS	NS		NS
	HES_{BAL}	0.01	NS	NS	
Extem-Alpha	RL		NS	0.03	<0.01
	GEL	NS		NS	NS
	HES_{SAL}	0.03	NS		NS
	HES_{BAL}	<0.01	NS	NS	
Extem-MCF	RL		NS	0.04	<0.01
	GEL	NS		NS	NS
	HES_{SAL}	0.04	NS		NS
	HES_{BAL}	<0.01	NS	NS	
Intem-CT	RL		NS	NS	NS
	GEL	NS		NS	NS
	HES_{SAL}	NS	NS		NS
	HES_{BAL}	NS	NS	NS	
Intem-CFT	RL		NS	0.01	<0.01
	GEL	NS		NS	NS
	HES_{SAL}	0.01	NS		NS
	HES_{BAL}	<0.01	NS	NS	

Continued

Table A1 *Continued*

		RL	GEL	HES_{SAL}	HES_{BAL}
Intem-Alpha	RL		<0.01	<0.01	<0.01
	GEL	<0.01		NS	<0.01
	HES_{SAL}	<0.01	NS		NS
	HES_{BAL}	<0.01	<0.01	NS	
Intem-MCF	RL		0.01	<0.01	<0.01
	GEL	0.01		NS	NS
	HES_{SAL}	<0.01	NS		NS
	HES_{BAL}	<0.01	NS	NS	
Natem-CT	RL		0.03	NS	NS
	GEL	0.03		NS	NS
	HES_{SAL}	NS	NS		NS
	HES_{BAL}	NS	NS	NS	
Natem-CFT	RL		NS	NS	NS
	GEL	NS		NS	NS
	HES_{SAL}	NS	NS		NS
	HES_{BAL}	NS	NS	NS	
Natem-Alpha	RL		NS	NS	NS
	GEL	NS		NS	NS
	HES_{SAL}	NS	NS		NS
	HES_{BAL}	NS	NS	NS	
Natem-MCF	RL		<0.01	0.02	<0.01
	GEL	<0.01		NS	NS
	HES_{SAL}	0.02	NS		NS
	HES_{BAL}	<0.01	NS	NS	

Table A2 Intergroup comparisons of 66% haemodilution with different volume expanders ($P<0.05$ was considered statistically significant). GEL, saline-based gelatin solution; RL, balanced-electrolyte lactated Ringer's solution; HES_{BAL}, balanced-electrolyte HES solution; HES_{SAL}, saline-based HES solution; ACT, activated clotting time; CFT, clot formation time; CR, clot rate; CT, coagulation time; MCF, maximum clot firmness; NS, not significant; PA, peak amplitude

		RL	GEL	HES_{SAL}	HES_{BAL}
ACT	RL		<0.01	NS	NS
	GEL	<0.01		0.03	<0.01
	HES_{SAL}	NS	0.03		NS
	HES_{BAL}	NS	<0.01	NS	
CR	RL		0.04	0.02	<0.01
	GEL	0.04		NS	NS
	HES_{SAL}	0.02	NS		NS
	HES_{BAL}	<0.01	NS	NS	
TTP	RL		0.01	NS	NS
	GEL	0.01		NS	NS
	HES_{SAL}	NS	NS		NS
	HES_{BAL}	NS	NS	NS	
PA	RL		<0.01	<0.01	0.01
	GEL	<0.01		NS	NS
	HES_{SAL}	<0.01	NS		NS
	HES_{BAL}	0.01	NS	NS	
Extem-CT	RL		NS	NS	NS
	GEL	NS		NS	NS
	HES_{SAL}	NS	NS		NS
	HES_{BAL}	NS	NS	NS	

Continued

Table A2 *Continued*

		RL	GEL	HES_{SAL}	HES_{BAL}
Extem-CFT	RL		0.01	<0.01	<0.01
	GEL	0.01		NS	NS
	HES_{SAL}	<0.01	NS		NS
	HES_{BAL}	<0.01	NS	NS	
Extem-Alpha	RL		<0.01	<0.01	<0.01
	GEL	<0.01		NS	NS
	HES_{SAL}	<0.01	NS		NS
	HES_{BAL}	<0.01	NS	NS	
Extem-MCF	RL		<0.01	<0.01	<0.01
	GEL	<0.01		NS	NS
	HES_{SAL}	<0.01	NS		NS
	HES_{BAL}	<0.01	NS	NS	
Intem-CT	RL		NS	<0.01	0.02
	GEL	NS		NS	NS
	HES_{SAL}	<0.01	NS		NS
	HES_{BAL}	0.02	NS	NS	
Intem-CFT	RL		NS	<0.01	<0.01
	GEL	NS		0.02	<0.01
	HES_{SAL}	<0.01	0.02		NS
	HES_{BAL}	<0.01	<0.01	NS	
Intem-Alpha	RL		<0.01	<0.01	<0.01
	GEL	<0.01		0.03	0.01
	HES_{SAL}	<0.01	0.03		NS
	HES_{BAL}	<0.01	0.01	NS	
Intem-MCF	RL		<0.01	<0.01	<0.01
	GEL	<0.01		NS	NS
	HES_{SAL}	<0.01	NS		NS
	HES_{BAL}	<0.01	NS	NS	
Natem-CT	RL		NS	NS	NS
	GEL	NS		NS	NS
	HES_{SAL}	NS	NS		NS
	HES_{BAL}	NS	NS	NS	
Natem-CFT	RL		NS	NS	0.02
	GEL	NS		NS	0.04
	HES_{SAL}	NS	NS		NS
	HES_{BAL}	0.02	0.04	NS	
Natem-Alpha	RL		NS	NS	NS
	GEL	NS		NS	NS
	HES_{SAL}	NS	NS		NS
	HES_{BAL}	NS	NS	NS	
Natem-MCF	RL		NS	NS	<0.01
	GEL	NS		NS	NS
	HES_{SAL}	NS	NS		NS
	HES_{BAL}	<0.01	NS	NS	

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