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Expressiveness of global coagulation parameters in dilutional coagulopathy

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Key points

- The effects of diluting whole blood samples with either saline or hydroxyethyl starch to mimic dilutional coagulopathy was measured.

- The type of diluent was not important except for thromboelastometry where starch solution impaired haemostasis more than saline.

- aPTT does not correlate with CF activity up to dilution of about 60%, whereas PTI at all dilutions shows close correlation with the PTI-dependent clotting factors.

Background. Plasma-free volume replacement in haemorrhage often results in dilutional coagulopathy. Prothrombin time index (PTI) and activated partial thromboplastin time (aPTT) are used for monitoring haemostasis but have not yet been clinically evaluated. Our aim was to investigate the effects of haemodilution on the course of global coagulation tests and clotting factors (CFs).

Methods. Blood samples from each of 10 volunteers were diluted with sodium chloride 0.9% (saline) or 6% hydroxyethyl starch 130/0.4 (HAES) by 30–80%. PTI, aPTT, CF, and the thrombelastometric parameters (ROTEM®) coagulation time (CT) and maximum clot firmness (MCF) were determined.

Results. Dilution-dependent CF decreased in an almost linear manner and was not influenced by the diluent. Critically low activities for CF of ~30% and a fibrinogen concentration <100 mg dl^{-1} were measured at dilutions of between 60% and 75%. Critically low CF activities of about 30% were indicated by a PTI of 35–40%. PTI and MCF decreased continuously, demonstrating a good correlation with CF activities and fibrinogen. aPTT and CT showed a linear course up to a dilution of 65–75% corresponding to CF activities of 30–40%. Thereafter, values became pathological. PTI and aPTT were not influenced by the type of diluent, whereas the diluents had profound differences on results of thromboelastometry.

Conclusions. PTI and MCF are useful for monitoring dilution and intervention points. aPTT and CT reflect intervention points when showing pathological values. The type of diluents does not seem to interfere with PTI and aPTT, but HAES impairs haemostasis in ROTEM® more profoundly than saline.

Keywords: clotting factors; coagulopathy; global coagulation tests; haemostasis

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Massive haemorrhage is primarily treated by administration of colloid and crystalloid fluids to maintain normovolaemia. Haemoglobin concentrations below 6–10 g dl^{-1} justify the transfusion of red packed cell units in case of inadequate tissue oxygenation.[1] This therapy regime, however, induces dilutional coagulopathy (DC) with the risk of microvascular bleeding,[2,3] and it is still speculative which minimal concentrations of clotting factors (CFs) are required to maintain sufficient haemostasis. Since haemorrhage is responsible for 30–40% of trauma mortality, a goal-orientated haemostatic therapy is of interest.[4] Most guidelines recommend starting substitution therapy when the plasma activity of factors reaches 30% or 40%.[5,6] It has been shown that a prolongation of activated partial thromboplastin time (aPTT) and prothrombin time (PT) to 1.5–1.8 above the normal mean value is correlated with an increased risk of clinical coagulopathy.[5,7] Therefore, it is good clinical practice to perform PT (s), prothrombin time index (PTI, %) or

international normalized ratio (INR), and aPTT (s) tests in order to monitor haemostasis. These tests are used as surrogate parameters for the coagulation cascade as a whole. However, PT, PTI or INR, and aPTT have neither been intended nor validated for monitoring-acquired DC. Furthermore, the correlation between PT/PTI and aPTT values and CF concentrations has not been investigated sufficiently. In the literature, it is speculated that CF activities of 20–40% would correspond to a PTI of 30–50% and factor activities of 50–60% to a PTI of 60–80%.[6]

Over the last few years, point-of-care methods, in particular thrombelastometry, have gained wide acceptance for monitoring of acquired perioperative coagulopathy. Compared with PT/PTI and aPTT, thrombelastometry is less time-consuming and yields additional information regarding clot firmness and stability.[8,9] However, the ability of thrombelastometry to predict bleeding risk before operation or postoperative hypercoagulopathy is controversial.[10–12]

It has been shown that colloid agents affect the coagulation profile more profoundly than saline.[13] [14] Surprisingly, the influence of plasma-free volume replacement with crystalloids and colloids on global coagulation parameters such as PTI and aPTT remains unclear.

Therefore, the aim of our *in vitro* study was to determine how useful PTI, aPTT, and the thrombelastometric parameters clotting time (CT) and maximum clot firmness (MCF) are to indicate decreasing CF activities in increasing dilutions of whole blood using saline 0.9% or 6% hydroxyethyl starch 130/0.4 (HAES).

Methods

Subjects

After institutional ethical approval and informed written consent, 30 ml of citrated blood (Monovette® citrate tubes, Sarstedt AG, Nuernbrecht, Germany) was obtained from 20 healthy staff members. All individuals (16 male and four female, age range 28–45 yr) had a normal history of coagulation, renal and hepatic function, and none had taken anticoagulant or antiplatelet medication for at least 10 days. PTI, aPTT, and all thrombelastometric measurements of undiluted blood samples were in the normal range.

Conduct of the study

Modified rotational thrombelastometry (ROTEM®, Pentapharm, Munich, Germany) was performed in citrated whole blood using the intrinsically (INTEM®) and the extrinsically (EXTEM®) activated tests. Quantitative and qualitative fibrinogen/fibrin analysis was done by FIBTEM® test, which selectively inactivates thrombocyte function. The parameters CT (s) and MCF (mm) were also determined. CT corresponds to the reaction time in the conventional thromboelastogram (TEG®); MCF refers to the maximal amplitude of the tracing and reflects the tensile strength of the thrombus. All reagents were purchased from Pentapharm. Before each measurement, the ROTEM® device was checked for proper functioning according to the manufacturer's recommendations. ROTEM® tests were performed at 37°C within 3 h after venepuncture. Undiluted whole blood samples were used for baseline measurements. DC was then simulated by diluting whole blood with sodium chloride solution 0.9% (saline) (Braun, Melsungen, Germany) ($n=10$) or 6% HAES (Fresenius Kabi GmbH, Bad Homburg, Germany) ($n=10$). Preliminary tests had shown that both saline and HAES had a negligible influence on pH in our setting. Additionally, both solutions are calcium-free to avoid preliminary coagulation start. Whole blood samples were diluted as follows: 0% (baseline), 30%, 40%, 50%, 60%, 70%, and 80%.

For the global coagulation tests, PTI and aPTT, and also for the analysis of all CFs, whole blood was centrifuged for 20 min at 4000*g* within 1 h after venepuncture to obtain cell-free plasma. Plasma was then immediately frozen at −40°C for subsequent analysis. CFs I, II, V, VII, VIII, IX, X, XI, and XII were determined using the ACL Top® System (Instrumentation Laboratory, Bedford, MA, USA). For all CFs,

measurements were undertaken using a one-stage clotting assay in individual factor deficient plasma (HemosIL™, diaPharma® Group Inc., West Chester, OH, USA). The Clauss method was used for determination of fibrinogen. Factor XIII was determined with a Behring Coagulation System BCS® (Dade Behring, Deerfield, IL, USA) using the Berichrom™ F XIII assay (Dade Behring). PTI and aPTT were measured using the RecombiPlasTin® and SynthASil® assay, respectively (HemosIL™, diaPharma® Group Inc.). Using these reagents, normal ranges for PT were 12–26 s (automatically converted to PTI 120–70%) and for aPTT 30–42 s.

Low and critical low activities of factors II, V, VII, VIII, IX, X, XI, XII, and XIII were defined as 40% and 30%, for fibrinogen as 150 and 100 mg dl^{-1} and for PTI as 60% and 40%, as described in the literature.[5] [6]

Statistical analysis

All data were tested for normal distribution using Shapiro–Wilk's normality test. Data are presented as mean [standard deviations (SD)]. Differences between the two intervention groups were analysed by the use of the unpaired Student's *t*-test and a *P*-value of <0.05 was considered to be statistically significant. Correlation coefficients were calculated using the Spearman rank order test. SigmaPlot® Version 11.0 (Systat Software, San Jose, CA, USA) was used for statistical analysis.

Results

The results of isovolaemic haemodilution are presented in Figures 1–5. A direct comparison of ROTEM® parameters between saline and HAES is depicted in Figure 6. All undiluted baseline values of ROTEMw parameters and CF activities were within normal ranges. The effects of haemodilution PTI and PTI-dependent CF activities II, VII, and X are shown in Figure 1. A parallel decrease with increasing dilution was seen for all three factors. There was a very close correlation between PTI and factor activities with a correlation coefficient of 1.0 (*P*<0.001) for both saline and HAES. At a dilution of 60%, all CFs had an activity of about 40% in both diluents with a corresponding PTI of 45% (6) in saline and of 54% (9) in HAES, respectively. At a dilution of 70%, the factors reached a remaining critically low activity of ∼30%. At this point, PTI was 34% (5) in saline and 40% (5) in HAES.

Figure 2 depicts the impact of haemodilution on aPTT and aPTT-depending CFs II, VIII, IX, and X. With both diluents, there was a dilution-dependent parallel decrease in activity. Factor VIII activity was persistently lower compared with the other factors. The critically low factor activities of 40% and 30% were reached at dilutions of 60% and 70%, respectively. Up to a dilution of 60%, aPTT did not differ from baseline values. Factor activities of only 40% still resulted in aPTT in the upper physiological range and only became pathological when factor activities of 30% were reached.

The CT in EXTEM® test and its corresponding CFs II, VII, and X are shown in Figures 3 and 6. All the CFs decreased homogeneously in a linear manner. In contrast, up to a dilution of
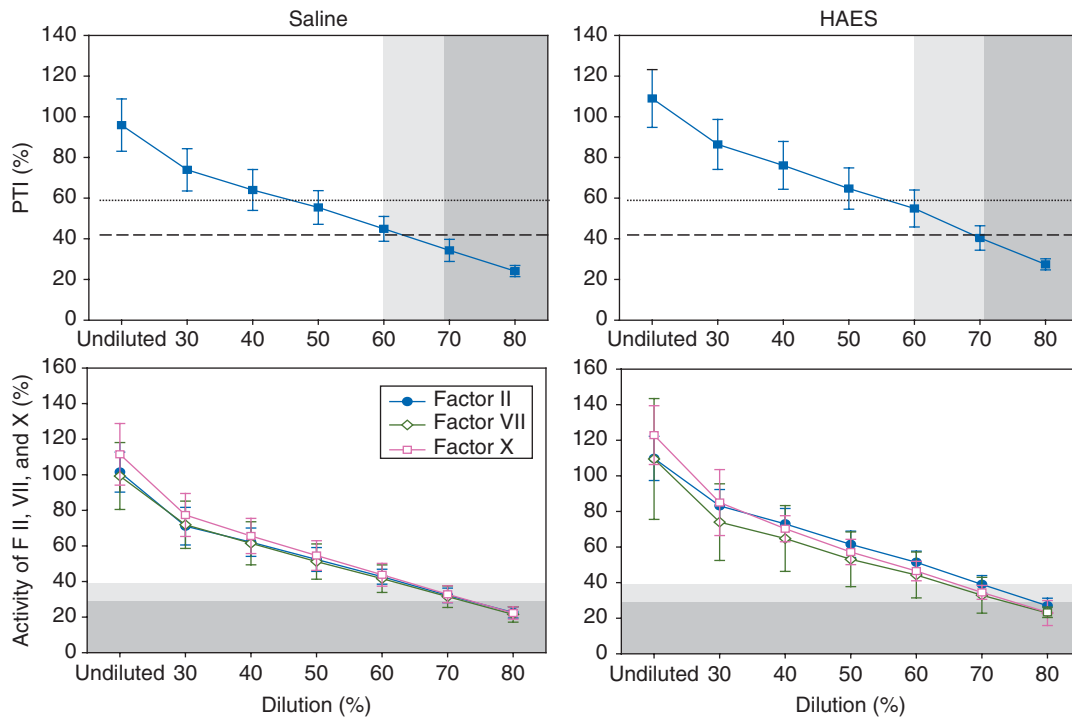
Fig 1 Influence of isovolaemic haemodilution with saline on HAES on PTI and its dependent CFs. Light grey areas indicate a CF activity of 30–40%, dark grey areas a CF activity of ≤30. The critical concentrations (light and dark grey areas) are mirrored to the global coagulation test PTI. The dotted line represents the lower limit of normal for PTI (60%), the dashed line indicates the critically low limit for PTI (40%). Values are means (SD).
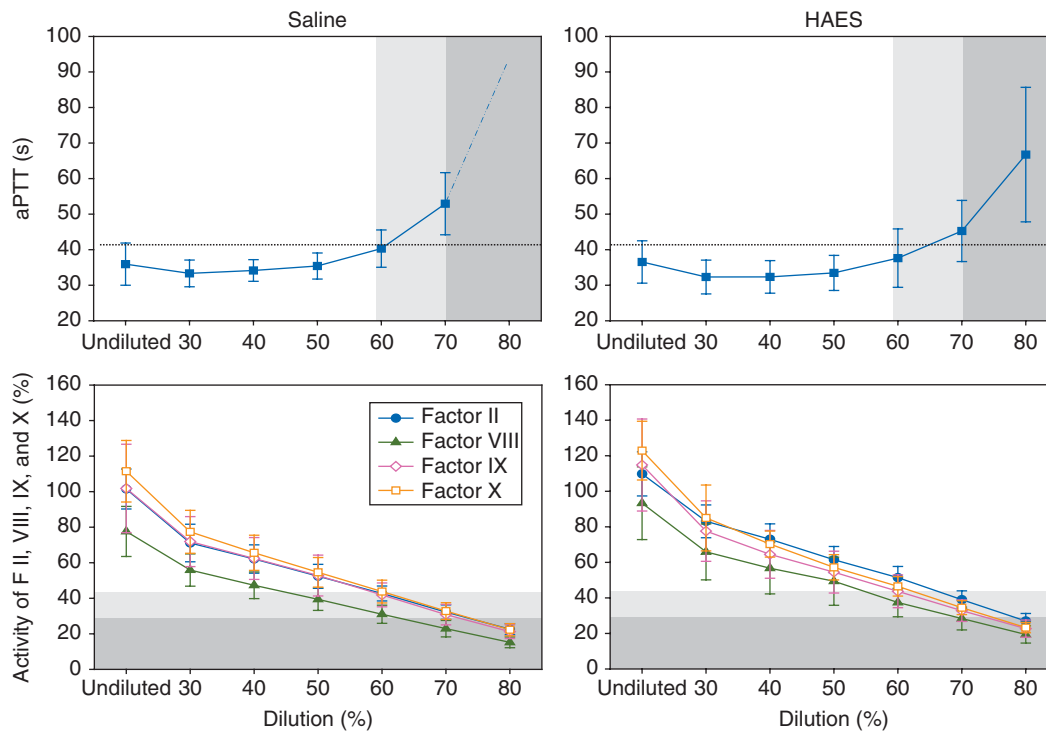

Fig 2 Influence of isovolaemic haemodilution with saline or HAES on aPPT and its dependent CFs. Light grey areas indicate a CF activity of 30–40%, dark grey areas a CF activity of ≤30. The critical concentrations (light and dark grey areas) are mirrored to the global coagulation test aPTT. The dotted line represents the upper normal limit for aPTT (42 s). Values are means (SD).
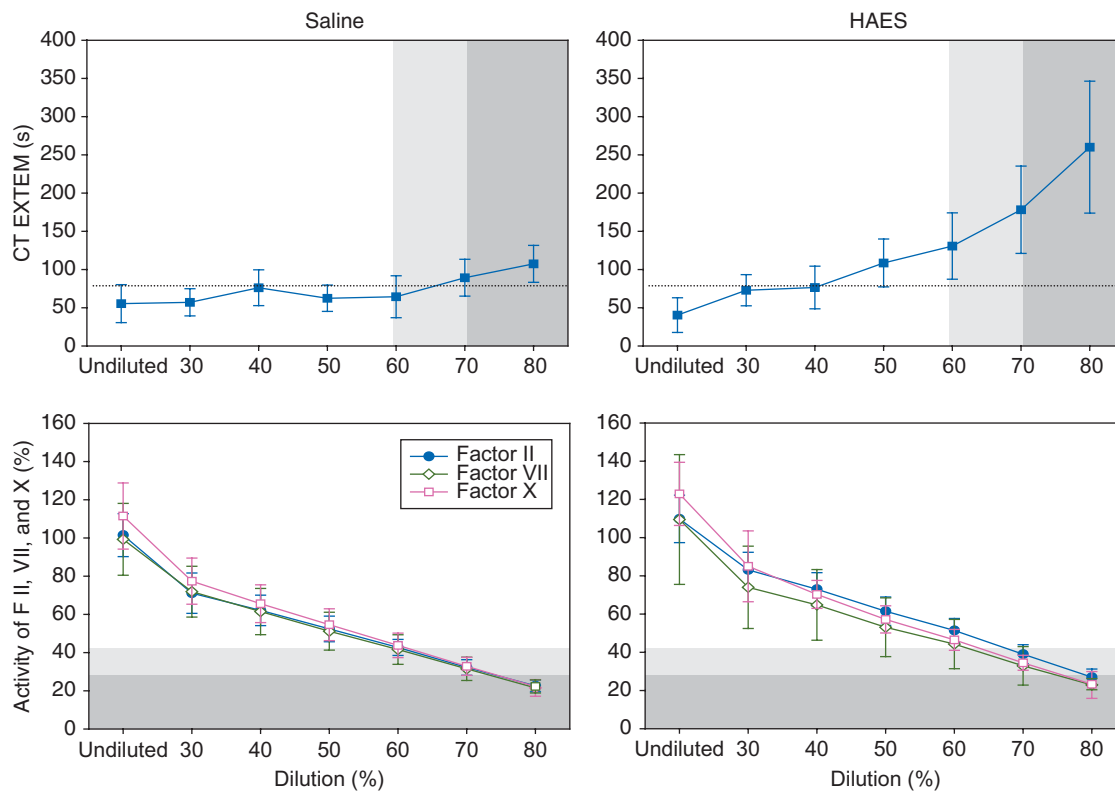
Fig 3 Influence of isovolaemic haemodilution with saline or HAES on CT EXTEM and its dependent CFs. Light grey areas indicate a CF activity of 30–40%, dark grey areas a CF activity of ≤30. The critical concentrations (light and dark grey areas) are mirrored to the CT EXTEM® test. The dotted line represents the upper normal limit for CT EXTEM® (79 s). Values are means (sd).

40% for HAES and 60% for saline CT EXTEM® values only differed slightly from baseline and were within the normal physiological range. At factor activities of 70%, the CT EXTEM® test (saline) showed pathological results. In contrast, at factor activities of 50%, CT EXTEM® measurements with HAES became pathological. As depicted in Figure 6, direct comparison of both groups revealed a profoundly steeper increase of CT EXTEM® with HAES compared with saline at dilution degrees of >50% (P<0.05).

Figure 4 describes the course of the CT INTEM® test with its dependent CFs II, VIII, IX, and X for both groups. Again, up to a dilution of 50% for HAES and 60% for saline, the CT INTEM® test remained almost unchanged compared with baseline, within the physiological range. At a dilution of 60% for HAES and 70% for saline, which corresponds to factor activities of about 40% and 30%, pathological test results were obtained. From 60–80% dilution, CT INTEM® in the HAES group shows a significantly steeper increase (P<0.05, not shown).

Figures 5 and 6 show the impact of fibrinogen concentration and factor XIII activity on MCF in INTEM®, EXTEM®, and FIBTEM® test for both saline and HAES. In all three tests, MCF decreased homogenously in a linear manner with dilution. Factor XIII also decreased with no relevant differences between diluents. Fibrinogen reached its lower

physiological value of 150 mg dl⁻¹ at a dilution of about 45% for saline and 55% for HAES. In blood diluted with saline, this corresponds to the lower range of 50 mm for MCF EXTEM® and INTEM®. At this point, MCF FIBTEM® still shows physiological test results. For blood diluted with HAES, the lower range of MCF EXTEM®, INTEM®, and FIBTEM® was reached at a dilution of 30–40%. The critical low level of fibrinogen of 100 mg dl⁻¹ is finally reached after a dilution of about 65% for saline and 75% for HAES, respectively. In both groups, this meant a dramatic reduction in clot strength for MCF INTEM® and EXTEM®. In the saline group, a fibrinogen concentration of 100 mg dl⁻¹ resulted in pathological values for MCF FIBTEM [8 (sd 3) mm], whereas at this fibrinogen concentration in the HAES group, there was no more relevant clotting at a dilution of >60% [1 (1) mm]. As depicted in Figure 6, there are profound differences in saline and HAES dilutions of 30–80% for MCF INTEM® and 40–80% for MCF FIBTEM®. In MCF EXTEM®, considerable differences were seen only at a dilution of 80%.

Discussion

DC occurs after massive haemorrhage in surgery and trauma and adversely affects both morbidity and mortality.[15][16] Therefore, it is of utmost importance to start coagulation
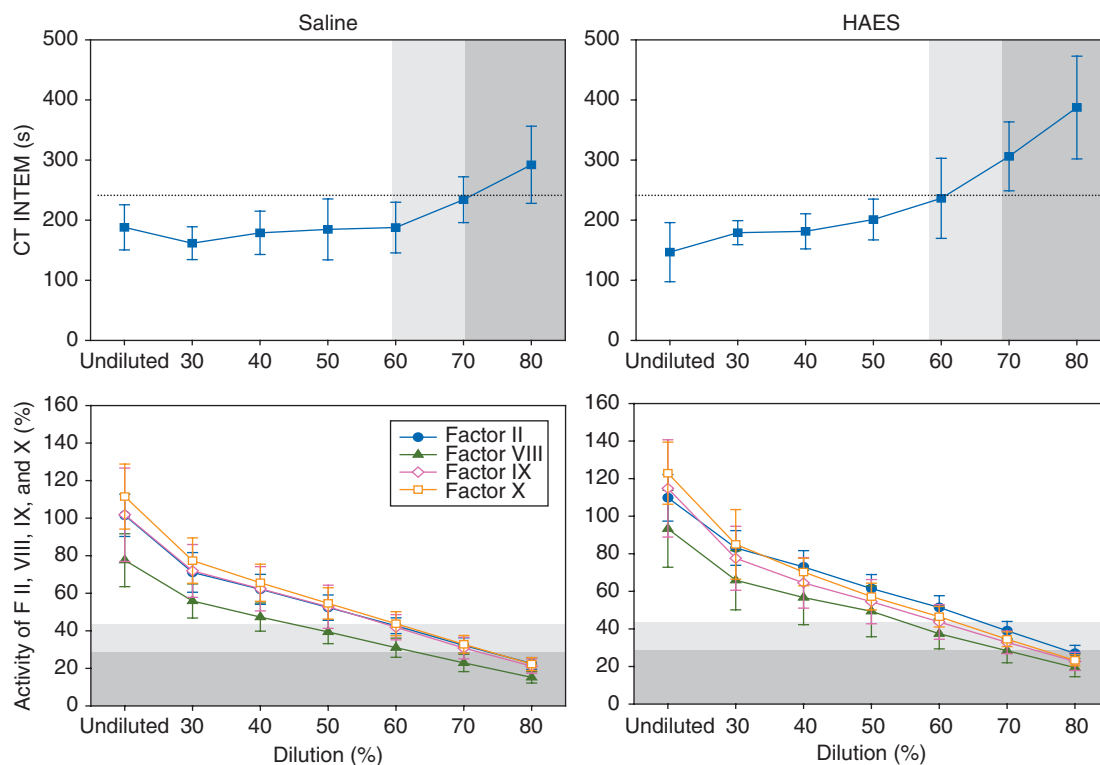
Fig 4 Influence of isovolaemic haemodilution with saline or HAES on CT INTEM and its dependent CFs. Light grey areas indicate a CF activity of 30–40%, dark grey areas a CF activity of ≤30. These critical concentrations (light and dark grey areas) are mirrored to the CT INTEM® test. The dotted line represents the upper normal limit for CT INTEM® (240 s). Values are means (sD).

therapy when the endogenous haemostatic system becomes insufficient.[15 16] Thus, monitoring of the coagulation system is mandatory.

PTI and aPTT are regarded to be poor estimators of bleeding risk in both elective and emergency settings.[17 18] Some authors, however, credit these tests with good sensitivity (≈80%) and specificity (≈70%) for identifying patients with an increased risk for postoperative bleeding in cardiac surgery, when tests were performed in the postoperative period.[11] Moreover, numerous current guidelines recommended the use of aPTT and PTI for monitoring a bleeding patient. However, these global coagulation tests have neither been designed nor been systematically validated, for monitoring or treating DC. Impact of haemodilution on PTI, aPTT, and CFs so far has only been cursorily investigated. The effect of a 40% isovolaemic haemodilution with Ringer's lactate on fibrinogen concentration and CFs VII, VIII, X, antithrombin, and protein C was assessed in rabbits.[19] Where there was a greater decrease in circulating pro-coagulant than anticoagulant activity, resulting in a haemodilution-mediated coagulopathy. Petroianu and colleagues[20] measured several coagulation factors and PTI and aPTT using crystalloid and colloidal fluids at dilution ratios of 10:4 (≈30%) and 10:10 (=50% dilution) in human subjects. Crystalloids caused effects on coagulation factors

which were explained by haemodilution alone. In contrast, the impact of hydroxyethyl starch and dextran solutions on CFs was less than that was expected by haemodilution alone. aPTT and PTI were influenced at a dilution of 50% by all diluents, but at a dilution of only 30% in crystalloids. The reason for this is unclear. Mittermayr and colleagues[14] examined the effects of fibrinogen concentrate on reversing coagulopathy *in vivo* after administration of either colloid or crystalloid solutions by performing ROTEM® analysis and measuring coagulation factors and global coagulation parameters. In their study, aPTT, PTI, and coagulation factors were more profoundly influenced by colloids compared with crystalloids. The kinetics of decay of CFs varies considerably in DC. Deficiency in fibrinogen develops much earlier than deficiency in factors II, V, and VII.[21] A previous study described prolongation of PT as the sentinel event in DC occurring early in bleeding operative patients. This finding was based on a computer simulation model generated from data from 44 patients.[22] Nevertheless, there is still a striking paucity of data in assessing the correlation of CFs and global coagulation tests under conditions of haemodilution.

Our data confirm a dilution-dependent homogenous decrease of all CFs and fibrinogen in both saline and HAES. PT/PTI and MCF showed a linear, CF-dependent decrease, thus reflecting ongoing haemodilution. Accuracy in PT/PTI
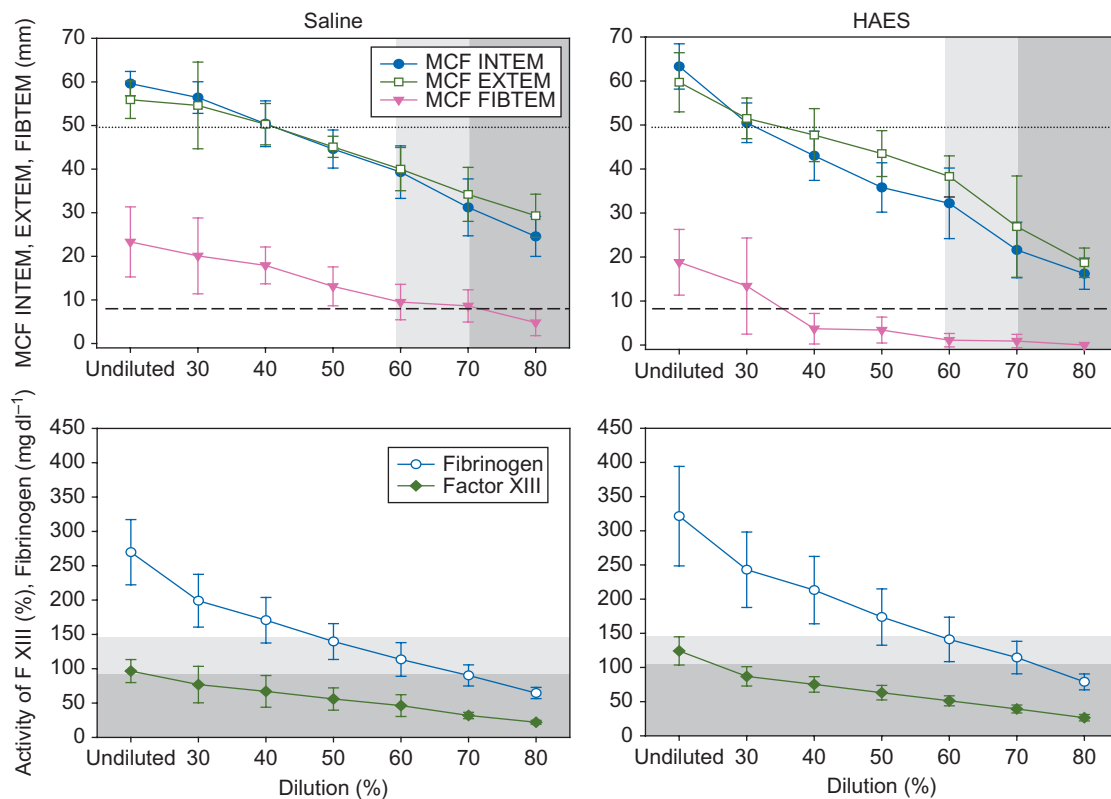
Fig 5 Influence of isovolaemic haemodilution with saline or HAES on factor XIII, fibrinogen, and MCF on ROTEM. Light grey areas indicate the lower range of fibrinogen (150 mg dl^{-1}), dark grey areas represent the critically low fibrinogen concentration (100 mg dl^{-1}). These concentrations are mirrored to the MCF INTEM®, EXTEM®, and FIBTEM® test, respectively. The dotted line represents the lower normal limit for MCF INTEM®/EXTEM® (50 mm), the dashed line indicates the lower limit for MCF FIBTEM® (9 mm). Values are means (SD).

tests was not affected by the two different diluents tested in our setting, whereas MCF measurements were sensitive to the diluent. HAES decreased MCF more profoundly than saline at corresponding dilutions. These findings are in accordance with previous studies.[13] [14]

In contrast, aPTT and CT did not correlate with the decrease in CFs up to dilutions of 60–70%. Thereafter, a sudden increase in the two global tests indicated critical low factor concentrations. Comparable with PT/PTI, aPTT likewise was not influenced by the kind of diluent used. In thrombelastometry, however, both diluents caused profound differences in CT measurements at dilutions of 60–80% for CT INTEM® and 50–80% for CT EXTEM®, respectively. The prolongation of CT by HAES rather might be ascribed to a compromised initiation of clot formation than to an impaired function of CFs of the intrinsic and extrinsic cascade.

As a consequence of our study, one might differentiate between progression and intervention tests of global coagulation. The progression tests PTI and MCF indicate both the time course of CF activity and an intervention threshold at critically low activities of CFs. The intervention parameters aPTT and CT only reflect an intervention threshold when leaving the normal range. Taking the normal upper limits for aPTT and CT as cut-off values, both tests seem to indicate

severely impaired coagulation at this point with remaining activities of CFs of about 30%, justifying acute coagulation therapy.[23]

In our setting, PTI values of 45% and 35% corresponded to a PTI-dependent CF activity of about 40% and 30%, respectively. Thus, PTI values seem to be a helpful parameter for estimation of the current PTI-dependent factor activity during bleeding, independently of the type of diluent.

In case of saline dilution, the lower physiological limit of 50 mm corresponds to a fibrinogen serum concentration of 150 mg dl^{-1} which is represented by an MCF FIBTEM® of 15 mm. Surprisingly, in the HAES group, 50 mm in MCF INTEM® and EXTEM® correspond to a fibrinogen concentration of >200 mg dl^{-1} which is accompanied by a critically low MCF FIBTEM® of only 4 mm. This antagonism can be explained by interactions of fibrinogen and HAES. Colloids lead to false high measurements of fibrinogen and induce defective fibrin polymerization.[24] [25] Thus, interpretation of test results for fibrinogen must take into consideration the kind of volume therapy of the patient.

Factor XIII deficiency is known to cause a prolongation of clot formation time (CFT) and a decrease in MCF in platelet-inhibited thromboelectrographic assays.[26] [27] CT on the contrary remains unaffected, since initiation of fibrin
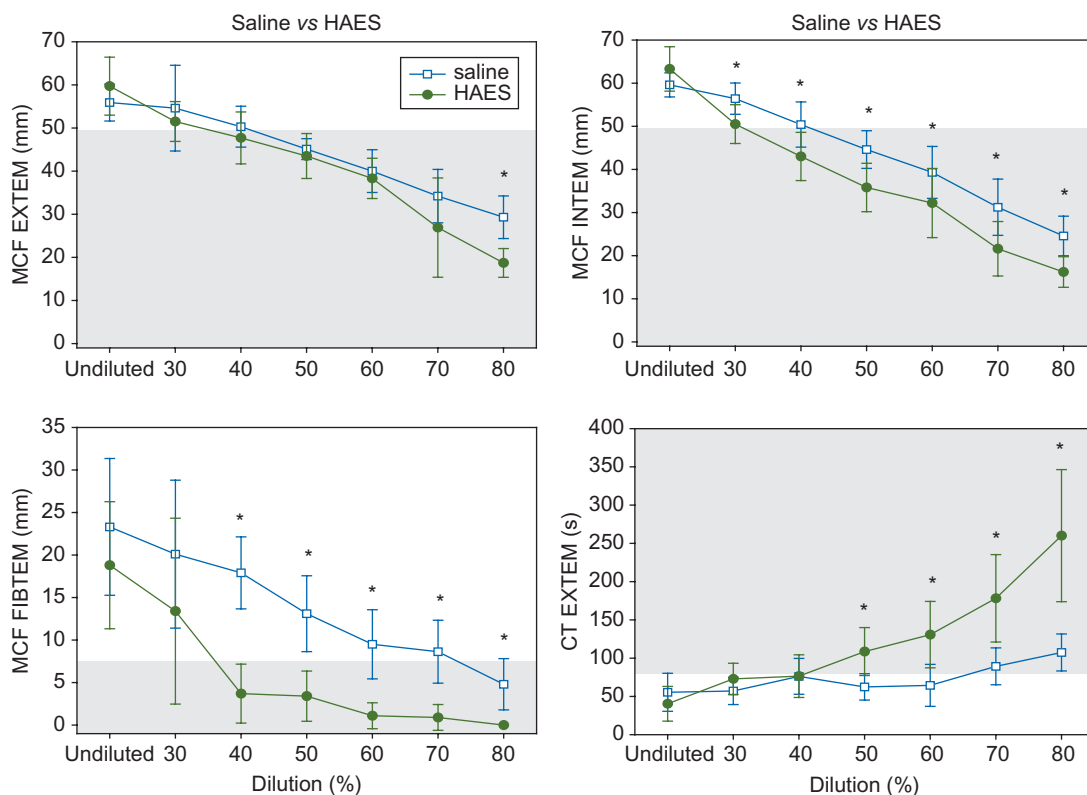
Fig 6 Influence of isovolaemic haemodilution with saline or HAES on ROTEM. Light grey areas represent critical ROTEM® parameters for MCF INTEM®/EXTEM® (50 mm), MCF FIBTEM® (9 mm), and CT EXTEM® (79 s). Values are means (SD). *$P<0.05$ corresponding degrees of dilution saline vs HAES.

aggregation is factor XIII-independent.[26] Some clinical data indicate increased bleeding in surgical patients showing a profound decrease in factor XIII.[28][29] Since the courses of factor XIII in saline and HAES in the results presented here do not differ substantially, the differences in MCF FIBTEM® are most likely attributable to the effects on fibrin polymerization by HAES.

The main limitation of our study is that our findings cannot directly be transferred to the clinical *in vivo* situation since *in vitro* tests lack the compensatory mechanisms.[30][31] In our *in vitro* setting, haemodilution alone was responsible for altered haemostatic potential, leading to a strictly linear and simultaneous decrease of all factors. *In vivo*, loss, consumption, and also dilution, of CFs occur in parallel, which might lead to differential changes of individual CFs. It has been shown that even a dilution of 30% may lead to fibrinogen deficiency.[32] Since crystalloids and colloids represent inhomogeneous pharmacological groups, our results may not be transferable to crystalloid and colloid volume replacement in general.[30] Furthermore, haemodilution with crystalloids might initially enhance coagulation at least *in vitro*, for reasons yet unknown.[33–35] Our findings that PTI and aPTT are not influenced by the kind of diluent are in contrast to results of Mittermayr and colleagues.[14] Their *in vivo* findings indicate a more profound change in aPTT and PTI for HAES and

gelatine compared to Ringer's lactate. However, the dilutions were unable to be definitely stated due to the complex experimental setting. Furthermore, it should be noted the differing resting time of colloids and crystalloids in the vascular system influencing coagulation tests.

In conclusion, our *in vitro* model demonstrated that PTI shows close correlation with the PTI-depending CF activities and is independent of the type of diluent used. MCF reflects fibrinogen concentration but other than PTI, MCF differs with the type of diluents used. In contrast, aPTT and CT do not correlate with CF activity up to a dilution of about 60%. CT, like MCF, is influenced by the diluent used. For all parameters, intervention points can be defined, at which critically low factor activities are reached.

Conflict of interest

None declared.

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