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Effect of progressive haemodilution with hydroxyethyl starch, gelatin and albumin on blood coagulation

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Summary

We have compared the effects of progressive (30% and 60%) *in vitro* haemodilution with hydroxyethyl starch (HES), gelatin (GEL) and albumin (ALB) with haemodilution using 0.9% saline in 96 patients by thrombelastography. Haemodilution with HES, GEL and ALB significantly ($P<0.05$) compromised coagulation time (k), angle α and maximal amplitude (MA), with HES having the most negative effect at 30% and 60% haemodilution ($P<0.05$). Haemodilution with saline significantly affected all variables of blood coagulation and clot lysis measured by thrombelastography, resulting in an increased coagulability at 30% haemodilution. To specifically assess the intrinsic effect of plasma expander molecules on blood coagulation and clot lysis, we analysed the difference between saline diluted blood (same degree of haemodilution) and plasma expander diluted blood. Prolongation of reaction time (r) was found for HES at 30% and 60% haemodilution and for ALB at 60% haemodilution and an increase in clot lysis by HES, GEL and ALB became evident. We conclude that HES, GEL and ALB compromised blood coagulation, while the maximum effect was found with HES. (*Br. J. Anaesth.* 1997; **78**: 684–689).

Key words

Blood, haemodilution. Measurement techniques, thrombelastography. Blood, coagulation. Blood, replacement.

In recent years there has been increasing awareness of the potential side effects of allogeneic blood products.[1] With increasing use of artificial plasma expanders, their influence on blood coagulation may become clinically relevant.[2] However, assessing the influence of various plasma expanders on blood coagulation is difficult because the observed effect may be a result of an intrinsic effect of the plasma expander molecules in addition to an effect of haemodilution on blood coagulation *per se*. Haemodilution may not necessarily compromise blood coagulation. In contrast, haemodilution with crystalloids may even increase blood coagulation as shown *in vitro*[3] and *in vivo*.[4–6] To standardize the degree of haemodilution and, in particular, to distinguish between the effect of plasma expanders and the effect of haemodilution *per se*, we have assessed *in vitro* the effect of hydroxyethyl starch, gelatin and albumin on blood coagulation compared with saline diluted blood samples using thrombelastography.

Patients and methods

With approval of the Ethics Committee, a 10-ml blood sample was collected from 126 patients undergoing various surgical procedures. In a pilot study (30 patients) blood was collected during induction of anaesthesia and in the main study (96 patients) blood was collected before induction. Exclusion criteria were known coagulation disorders, prothrombin time less than 85%, non-steroidal anti-inflammatory agents (NSAID) within 24 h before surgery, oral anticoagulation, acetylsalicylic acid (ASA) within 5 days before operation, known liver diseases or increased plasma concentrations of aspartate aminotransferase (AST >50 iu litre^{-1}) or alanine aminotransferase (ALT >50 iu litre^{-1}), and known renal diseases or increased creatinine concentration (creatinine >120 μmol litre^{-1}). Standard thrombosis prophylaxis of low molecular .weight heparin administered s.c. (3000 IE, Sandoparin) the evening before surgery was allowed.

Blood coagulation was assessed by thrombelastography (CTEG #3000, Haemoscope, Morton Grove, IL, USA). Whole blood (0.36 ml) is placed in a cuvette which is rotated back and forth. A piston is suspended in the blood, and as coagulation proceeds, fibrin strands form between the walls of the cuvette and the piston. The piston thus becomes increasingly coupled to the motion of the cuvette, and hence the shearing elasticity of the evolving blood clot is detected to yield the TEG trace.[3 6] This TEG trace is described by specific variables. Reaction time (r) is the interval between the start of the recording until an amplitude of 2 mm is reached (normal value 12.0 (2.3) mm). It reflects the function of the coagulation factors. Coagulation time (k)

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is defined as the time interval from the end of *r* until the amplitude of the TEG tracing reaches 20 mm (normal value 4.2 (1.6) mm); *k* is influenced not only by coagulation factors but also by fibrinogen and the number and function of platelets. Maximum amplitude (MA) of the TEG tracing reflects clot strength as a function of platelets and fibrinogen (normal value 63.5 (4.5) mm). The angle α is formed by the slope of the TEG tracing from the *r* to the *k* value and is, similarly to MA, influenced by the function of platelets and fibrinogen (normal value 60.2 (6.7) °). Clot lysis can be described by the clot lysis index (CLI), defined as the amplitude of the TEG trace at 60 min after MA (A60) divided by MA (CLI = A60/MA × 100, %) or by Ly30 and Ly60.[7] The fibrinolysis indexes, Ly30 and Ly60, computed by the current TEG device, are defined as the percentage reduction of the computed area under the curve 30 and 60 min after reaching MA (normal value for Ly30 < 7.5% and Ly60 < 15%). All normal values apply to plastic cups and plastic pins with 1% celite activation (CTEG manual, Haemoscope Corporation, 1990).

The following volume expanders were investigated: hydroxyethyl starch 200 000/0.5 (HES) (Isohes 6%, Laevosan International AG, Zurich, Switzerland), succinylated gelatin (GEL) (Physiogel 4%, Braun Medical, Emmenbrücke, Switzerland) and 5% human serum albumin (ALB) (ZLB Zentrallaboratorium Blutspendedienst SRK, Bern, Switzerland). In pilot studies (30 patients), we found that sodium bicarbonate and $CaCl_2$ had to be added to all volume expanders and also to 0.9% saline such that physiological pH and ionized Ca^{2+} values resulted after *in vitro* haemodilution. However, the amount of sodium bicarbonate and $CaCl_2$ was not the same for different volume expanders. Thus we defined (pre-haemodilution) ranges for pH and ionized Ca^{2+} for all volume expanders and 0.9% saline which resulted in physiological pH and physiological ionized Ca^{2+} after *in vitro* haemodilution. The following ranges were found: HES (pH 6.66–6.92; Ca^{2+} 1.22–1.29 mmol litre^{-1}), GEL (pH 7.28–7.40; Ca^{2+} 1.24–1.29 mmol litre^{-1}), ALB (pH 7.10–7.24; Ca^{2+} 1.32–1.38 mmol litre^{-1}) and saline (pH 6.88–7.45, Ca^{2+} 1.18–1.24 mmol litre^{-1}). Adjusting pH and calcium concentration of all volume expanders and 0.9% saline *in vitro* before haemodilution is important because *in vitro* there are no effective homeostatic mechanisms preventing relevant changes in pH and electrolyte concentrations caused by haemodilution with crystalloids or colloids. Without such a correction blood coagulation would have been impaired by low ionized Ca^{2+} and low pH.[8–11]

Blood samples from 96 patients were allocated randomly to one of six groups ($n = 16$) to be haemodiluted with HES, GEL or ALB, either by 30% or 60%. In each patient, blood coagulation was assessed simultaneously in native blood, after haemodilution with a volume expander and also after haemodilution to the same degree with saline (see below).

Foaming or frothing was prevented carefully during blood sampling. The first 2 ml were discarded and then 8 ml were sampled in polypropylene syringes. With reverse pipetting the appropriate amount of blood was filled into prewarmed (37.0 °C) polypropylene tubes containing volume expander or saline to prevent cooling of the blood volume expander mixture which might compromise blood coagulation.[11 12] Mixing was performed carefully by filling and half-emptying the pipette five times. The same procedure was performed with native blood so as to prevent activation of the coagulation system through handling. Three minutes after mixing, 1 ml of native blood, 1 ml of volume expander diluted blood and 1 ml of saline diluted blood were filled into tubes containing 1% celite.[13] One minute later (4–5 min after blood withdrawal), 360 μl were pipetted into the thrombelastographic cups. Exactly 6 min after withdrawal of blood, thrombelastography was started.[7] Plastic pins and plastic cups used were put in the TEG at least 10 min before starting the assay. The temperature of the TEG was maintained at 37.0 °C. The remainder of the native and diluted blood was used for blood-gas, electrolyte and haemoglobin measurements (BGElectrolytes and CO-Oximeter, Instrumentation Laboratory, Lexington, MA, USA and Electrolyte 8 Analyser, Nova Biomedica, Waltham, MA, USA).

Unpaired *t* tests were used to compare patient characteristics (Statview 4.02, Abacus Concepts, Inc., Berkeley, CA). Changes caused by *in vitro* haemodilution were analysed by repeated measures analysis of variance (ANOVA) with Greenhouse–Geisser correction (Superanova 1.11, Abacus Concepts, Inc., Berkeley, CA, USA). When this overall ANOVA resulted in a significant haemodilution and plasma expander effect, respectively, *post hoc* paired and unpaired *t* tests with Bonferroni correction were performed. Data are presented as mean (SD) and mean (SEM) as appropriate. $P < 0.05$ was considered statistically significant.

Results

Three were no differences in the HES, GEL and ALB groups in patient characteristics (table 1) or baseline data (table 2, figs 1, 3).

Haemodilution with HES, GEL, ALB and saline resulted in a similar decrease in haemoglobin concentration (table 2). Ionized Ca^{2+} concentration was stable during haemodilution in the HES and GEL groups but decreased minimally in the ALB group at

Table 1 Characteristics of patients in the HES, GEL and ALB groups. Data are mean (SD or range). There are no significant differences between groups. LMWH = low molecular weight heparin

	HES ($n = 32$)	GEL ($n = 32$)	ALB ($n = 32$)
Age (yr)	53.1 (18–74)	51.6 (19–80)	52.3 (21–79)
BMI (kg m^{-2})	25.8 (3.6)	25.4 (3.2)	25.5 (3.7)
Sex (F/M)	11/21	7/25	9/23
Creatinine (μmol litre^{-1})	90 (11)	93 (10)	89 (20)
Platelet count (10^3 μl^{-1})	235 (61)	224 (46)	225 (58)
LMWH administered	18 of 32	18 of 32	19 of 32
Incidence of carcinoma	3 of 32	5 of 32	6 of 32

Table 2 Ionized Ca^{2+} concentration (Ca^{2+}), pH (pH) and haemoglobin concentration (Hb) after haemodilution (HD) with HES, GEL, ALB and saline. Data are mean (SD). Significantly different *($P<0.05$) from native blood

	HES ($n=16$)	GEL ($n=16$)	ALB ($n=16$)	Saline ($n=48$)
Hb (g dl^{-1})				
0% HD	14.07 (1.55)	14.35 (1.39)	14.55 (1.20)	14.32 (1.39)
30% HD	9.84 (1.19)*	10.21 (1.00)*	10.31 (0.82)*	10.16 (1.01)*
60% HD	5.76 (0.63)*	5.67 (0.55)*	5.88 (0.54)*	5.85 (0.58)*
Ca^{2+} (mmol $litre^{-1}$)				
0% HD	1.23 (0.04)	1.24 (0.04)	1.23 (0.04)	1.23 (0.04)
30% HD	1.23 (0.03)	1.25 (0.04)	1.19 (0.03)*	1.22 (0.03)*
60% HD	1.24 (0.02)	1.25 (0.03)	1.19 (0.04)*	1.21 (0.03)*
PH				
0% HD	7.43 (0.03)	7.43 (0.02)	7.42 (0.03)	7.43 (0.03)
30% HD	7.42 (0.02)*	7.41 (0.02)*	7.42 (0.03)	7.41 (0.02)*
60% HD	7.40 (0.03)*	7.42 (0.02)*	7.41 (0.05)	7.41 (0.03)*

30% and 60% haemodilution (table 2). pH was stable during haemodilution with ALB and decreased minimally during haemodilution with HES and GEL (table 2). Also, during haemodilution with saline, the ionized Ca^{2+} concentration and pH decreased minimally at 30% and 60% haemodilution (table 2). However, ionized Ca^{2+} concentration and pH were within the physiological range after haemodilution with all volume expanders and saline (table 2).

Compared with native blood, progressive haemodilution with HES, GEL and ALB did not affect reaction time (*r*) (fig. 1A) but compromised the rapidity of clot formation, as manifested by an increase in coagulation time (*k*) and a decrease in the clot formation rate (angle α) (fig. 1B, C). With HES, this was significant at 30% haemodilution; with GEL and ALB, significant changes were observed only at 60% haemodilution. The strength of the clot (MA) was decreased by HES, GEL and ALB at 30% and 60% haemodilution (fig. 1D). Thus HES had the most negative effect on *k*, angle α and MA. Clot lysis at 30 or 60 min was not affected by haemodilution (fig. 1E, F). No individual patient had pathological clot lysis caused by haemodilution, that is Ly30 > 7.5 % or Ly60 > 15%.

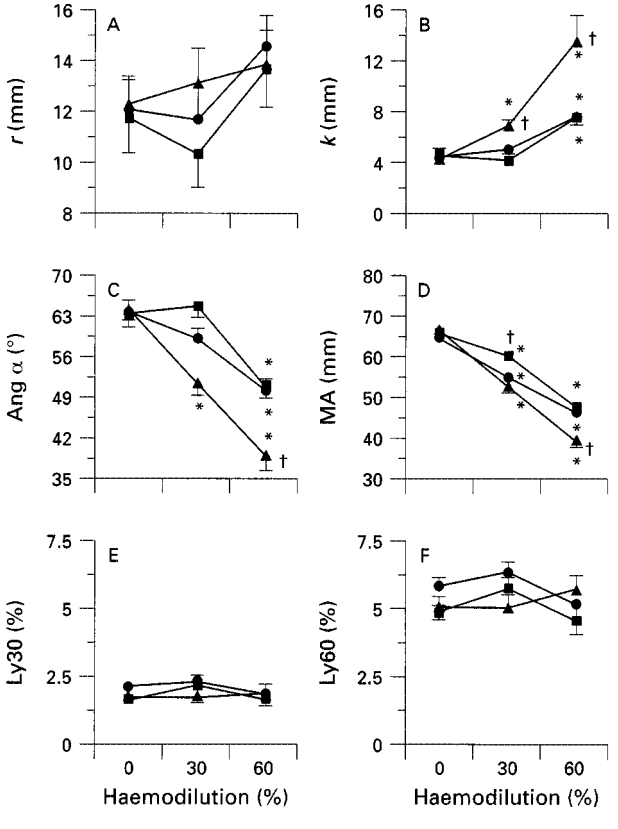

Figure 1 Effect of progressive (30%, 60%) *in vitro* haemodilution with hydroxyethyl starch (▲), gelatin (●) and albumin(■) on blood coagulation, as assessed by thrombelastography: reaction time (*r*) (A), coagulation time (*k*) (B), angle α (Ang α) (C), maximal amplitude (MA) (D), lysis after 30 min (Ly30) (E) and lysis after 60 min (Ly60) (F). Data are mean (SEM). Significantly different (*$P<0.05$) from native blood; significantly different (†$P<0.05$) from other groups.

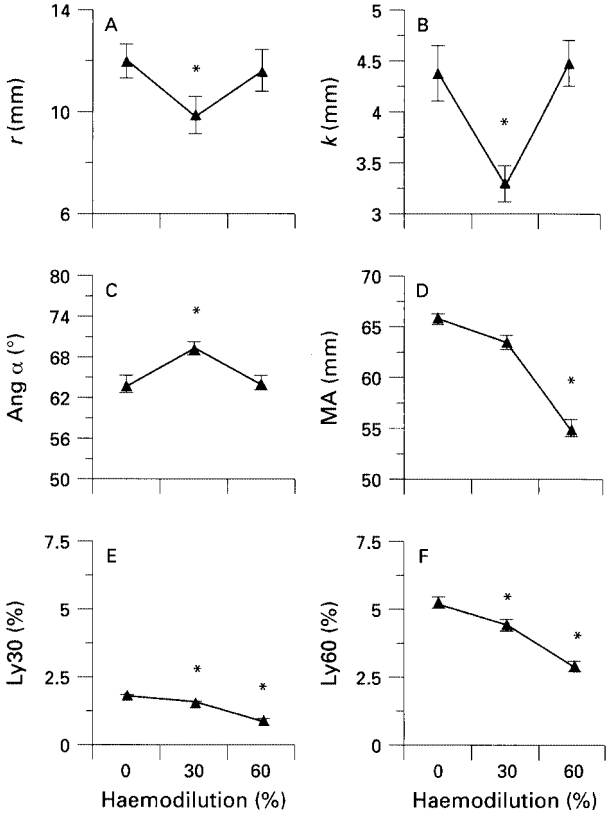

Figure 2 Effect of progressive (30%, 60%) *in vitro* haemodilution with saline (▲) on blood coagulation as assessed by thrombelastography: reaction time (*r*) (A), coagulation time (*k*) (B), angle α (Ang α) (C), maximal amplitude (MA) (D), lysis after 30 min (Ly30) (E) and lysis after 60 min (Ly60) (F). Data are mean (SEM). Significantly different (*$P<0.05$) from native blood.

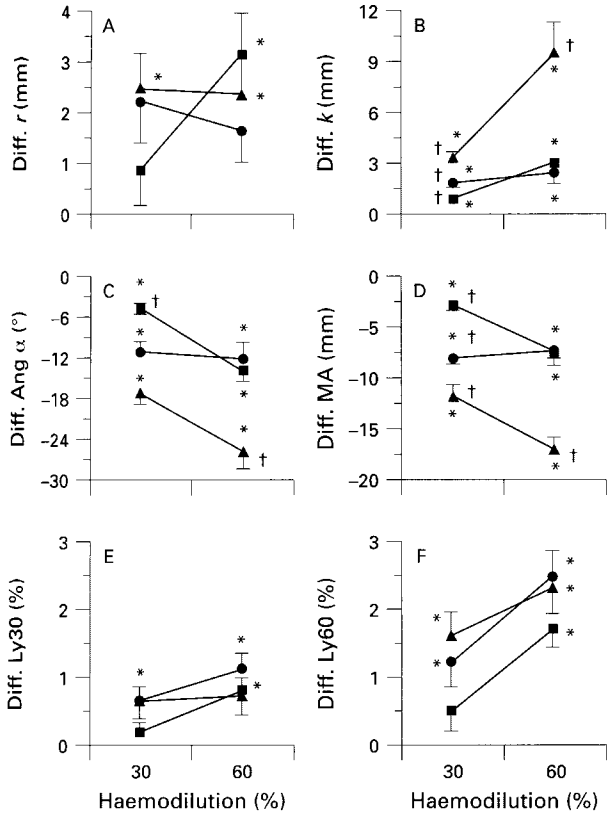

Figure 3 Effect of progressive (30%, 60%) *in vitro* haemodilution with hydroxyethyl starch (▲), gelatin (●) and albumin(■) on blood coagulation as assessed by thrombelastography in comparison with haemodilution with 0.9% saline, expressed as absolute difference (Diff.) of values (expander minus saline): reaction time (Diff. *r*) (A), coagulation time (Diff. *k*) (B), angle α (Diff. Ang α) (C), maximal amplitude (Diff. MA) (D), lysis after 30 min (Diff. Ly30) (E) and lysis after 60 min (Diff. Ly60) (F). Data are mean (SEM). Significantly different (*$P<0.05$) from 0; significantly different (†$P<0.05$) from other groups.

To specifically assess the intrinsic effect of plasma expander molecules on blood coagulation, we first analysed the effect of haemodilution with saline *per se* (compared with native blood). At 30% haemodilution, reaction time (*r*) and coagulation time (*k*) decreased and clot formation rate (angle α) increased. At 60% haemodilution, all three variables recovered (fig. 2A–C). MA was compromised at 60% haemodilution, and clot lysis at 30 and 60 min (Ly30 and Ly60) showed progressive decrease with increasing haemodilution (fig. 2D–F).

The intrinsic effect of plasma expander molecules on blood coagulation may thus be assessed by analysing the difference between saline diluted blood (same degree of haemodilution) and plasma expander diluted blood. Haemodilution with HES resulted in a longer reaction time (*r*) at 30% and 60% haemodilution compared with saline haemodilution, and ALB resulted in a longer reaction time (*r*) at 60% haemodilution (fig. 3A). Coagulation time (*k*) was prolonged by haemodilution with HES, GEL and ALB at 30% and 60% haemodilution compared with saline haemodilution (fig. 3B), and also clot formation rate (angle α) was less during 30% and 60% haemodilution using HES, GEL and ALB than during haemodilution with saline (fig. 3C). Furthermore, the decrease in the strength of the clot, that is the decrease in MA, was more pronounced during 30% and 60% haemodilution with HES, GEL and ALB compared with haemodilution using saline (fig. 3D). HES had the most negative effect on *k*, angle α and MA. Haemodilution with GEL and ALB resulted in increased clot lysis at 30 min (Ly30) in comparison with haemodilution with saline (fig. 3E), and clot lysis at 60 min (Ly60) was increased more during haemodilution with HES, GEL and ALB compared with haemodilution with saline (fig. 3F).

There were no differences between patients with and without cancer, either at baseline or at any degree of haemodilution with any volume expander tested (data not shown).

Discussion

We have demonstrated that blood coagulation was compromised by *in vitro* haemodilution using hydroxyethyl starch, gelatin or albumin. However, hydroxyethyl starch had the most pronounced effect. Also, when the effect of haemodilution with saline is considered, hydroxyethyl starch, gelatin and albumin also increased clot lysis.

Because of increasing awareness of the potential side effects of allogeneic blood products, volume expanders are used increasingly to treat surgical blood loss.[1] Thus the side effects of volume expanders such as interference with blood coagulation may become clinically relevant. In this study we found that while hydroxyethyl starch had the most pronounced effect, *all* volume expanders showed qualitatively similar effects, namely a marked decrease in the strength of the clot (MA) and rapidity of clot formation (angle α), and also an increase in coagulation time (*k*) (figs 1, 3). This pattern is compatible with platelet dysfunction[14] and with previous reports of a decrease in all factor VIII moieties produced by hydroxyethyl starch and dextran infusions,[15–20] which might result in platelet dysfunction.[21] However, the decrease in factor VIII was not observed when dextran or hydroxyethyl starch was added *in vitro*.[17 22] These *in vitro* studies, however, were performed in citrated plasma to prevent clotting and thus may not be representative of the present study because blood coagulation in this study was not inhibited but progressed during thrombelastography measurement. When blood coagulation is not inhibited, hydroxyethyl starch and dextran solutions can precipitate coagulation factors also *in vitro*[23] and thus compromised blood coagulation during haemodilution might be caused by reduced clotting factor activity, in particular factor VIII.

We found significant impairment of blood coagulation with hydroxyethyl starch but also with albumin and gelatin (figs 1, 3). Thus during profound haemodilution, blood coagulation may be compromised irrespective of the volume expander used. Indeed, recent data suggest that abnormal haemostasis may develop before compromise of global tissue oxygen delivery and consumption.[2]

The effects of plasma expanders on clot lysis have

not been investigated fully. In the presence of hydroxyethyl starch, faster transformation of fibrinogen to fibrin has been shown.[24 25] This fibrin was shown to be less stable as evidenced by a shortened urokinase-activated clot lysis time suggesting enhanced fibrinolysis.[24 25] Also, gelatin has been shown to be incorporated into developing blood clots and thus potentially interfere with fibrin polymerization.[26] Similarly, we also found progressively augmented clot lysis for all three plasma expanders compared with saline. This effect, however, was apparent only when the influence of haemodilution with saline *per se* on clot lysis was taken into account (fig. 3). Despite this augmentation, clot lysis was, on average, still within normal ranges and thus may be of limited clinical relevance.

Interestingly, moderate haemodilution with crystalloids appeared to augment blood coagulation (fig. 2) which is in keeping with earlier *in vitro*[3] and *in vivo* observations.[4–6] Recently, Ruttmann, James and Viljoen[3] described a hypercoagulable state at 20% haemodilution with saline, as evidenced by a shortened reaction time (*r*) and an increased rapidity of clot formation (*k* and angle α). At 30% haemodilution with saline, we also found a shortened reaction time (*r*) and increased rapidity of clot formation (*k* and angle α) (fig. 2). At 30% haemodilution with gelatin, however, we did not observe such an augmentation of blood coagulation (fig. 1) compared with Ruttmann, James and Viljoen,[3] who also described a shortened reaction time (*r*) and increased rapidity of clot formation (*k* and angle α) at 20% haemodilution with gelatin. The difference might be explained by the more profound degree of haemodilution produced in this study. Interestingly, although we did not observe a hypercoagulable state at 30% haemodilution with gelatin, *k* and angle α were unchanged and became compromised only at 60% haemodilution, indicating that the degree of haemodilution may be crucial for changes in blood coagulation during haemodilution.

The mechanisms by which blood coagulation is facilitated during moderate haemodilution with saline are largely unknown. One might hypothesize, however, that the anticoagulant activity of blood may be compromised more than the procoagulant activity during moderate haemodilution, resulting in a hypercoagulable state. With advanced haemodilution also, procoagulant activity may become diluted and the net result at profound haemodilution is compromised blood coagulation, as observed in this study at 60% haemodilution (fig. 2). Interestingly, we also found reduced clot lysis during haemodilution with saline (fig. 2). This effect was most prominent at profound haemodilution, that is at 60% haemodilution with saline. Further studies are necessary to elucidate these findings.

It is difficult to extrapolate our findings to clinical practice. Intraoperative use of volume expanders such as albumin and gelatin may compromise blood coagulation to a lesser extent than hydroxyethyl starch. However, in the postoperative period, a period characterized by a hypercoagulable state, hydroxyethyl starch may be beneficial in reducing the risk of thrombosis.[27]

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