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CLINICAL INVESTIGATIONS

In vivo effect of haemodilution with saline on coagulation: a randomized controlled trial[†‡]

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Background. Previous studies have shown that 10–30% haemodilution with crystalloid may induce a hypercoagulable state demonstrable by using the Thrombelastograph® (TEG). While most are *in vitro* studies, the few *in vivo* studies are limited by confounding surgical or 'environmental' factors. We conducted this randomized controlled study to evaluate the coagulation changes associated with *in vivo* haemodilution.

Methods. Twenty patients undergoing major hepatobiliary surgery were randomly allocated to one of two study groups. Group H (n=10) had 30% blood volume withdrawn over 30 min and replaced with saline. Group C (n=10) did not have any blood withdrawn. Blood samples were taken in both groups at 10, 20 and 30 min. Native TEG, complete blood count, coagulation profile, fibrinogen, antithrombin III, protein C and thrombin–antithrombin complex concentrations were measured.

Results. Compared with Group C, Group H patients had significantly greater shortening of r-time at 30 min (–30% *vs* +36%), greater shortening of k-time at all time points (–36% *vs* +17% at 10 min; –37% *vs* +44% at 20 min; –45% *vs* +49% at 30 min), and greater widening of α at 30 min (+71% *vs* +4%). The decrease in antithrombin III and other natural procoagulants and anticoagulants closely followed that of haematocrit, with the exception of thrombin–antithrombin complex.

Conclusion. *In vivo* haemodilution of up to 30% with saline can induce a hypercoagulable state. The mechanism remains unclear as disproportionate dilution of natural anticoagulants was not detected. Thrombin–antithrombin complex concentration remained stable despite haemodilution in Group H, which may suggest increased thrombin generation.

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Acute haemodilution of 20–30% with crystalloid has been shown to induce a hypercoagulable state demonstrable using the Thrombelastograph® (TEG). The characteristic changes include shortening of r-time and k-time, which indicates accelerated rate of clot formation, as well as widening of the angle (α), which indicates accelerated rate of clot stiffening. Most studies demonstrating this effect were performed using *in vitro* haemodilution.[1–6] Only two studies have provided evidence that hypercoagulability induced by acute crystalloid haemodilution can also be demonstrated *in vivo*.[7 8] In one of these studies, we demonstrated that in patients undergoing major surgery, only those who had considerable blood loss and haemodilution will develop significant hypercoagulability as measured on TEG.[7] However, there are obvious limitations to attributing all the changes we observed in the previous

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study to haemodilution *per se*. For example, tissue damage is likely to be more extensive in operations involving greater blood loss, and this may induce a more significant hypercoagulability response. Others have demonstrated the development of hypercoagulability *in vivo* in healthy volunteers receiving only an intravenous infusion of 1 litre normal saline.[8] Their findings have strengthened the hypothesis of the development of a hypercoagulability state with mild haemodilution.

However, a recent study reported that a hypercoagulable state might also be induced in patients during preparation for regional anaesthesia *per se* without administration of any drugs or intravenous fluids.[9] This suggested that some of the changes observed in previous *in vivo* studies could be attributable to confounding factors such as patient anxiety or insertion of intravenous lines. We therefore conducted this randomized controlled study to investigate the effect of up to 30% acute crystalloid haemodilution on coagulation. The design of the study will enable us to eliminate the effect of any confounding factors. We also measured the changes in various procoagulant and anticoagulant markers to delineate the mechanism underlying any changes observed. This includes measuring changes in fibrinogen, antithrombin III (AT III) and protein C concentrations, to detect any disproportionate dilution between procoagulants and anticoagulants. We also measured the concentration of thrombin–antithrombin complex to detect any increase in thrombin generation.

Methods

With institutional Ethics Committee approval and written informed consent, we recruited into the study ASA I–III patients undergoing major elective hepatobiliary surgery. These patients were scheduled to have intraoperative collection of autologous blood and acute isovolaemic haemodilution. We excluded patients with known haemostatic disorders, patients taking medication known to interfere with haemostasis, including oral contraceptives, and patients with severe anaemia, or cardiovascular or respiratory disease.

Eligible patients were randomly allocated to one of two study groups: haemodilution group (Group H) and control group (Group C). In both groups, general anaesthesia was induced with fentanyl 1.5–2 $\mu g\ kg^{-1}$, thiopental 3–4 $mg\ kg^{-1}$ and atracurium 0.5 $mg\ kg^{-1}$. The patient's trachea was intubated and the lungs ventilated with oxygen/air/isoflurane. Immediately after induction of anaesthetic, a 14 or 16G peripheral cannula (Angiocath, Becton Dickinson, Mexico), a right internal jugular cannula (Angiocath, Becton Dickinson, Mexico) and an arterial cannula (Insyte, Becton Dickinson, USA) were inserted. Autologous blood collection was performed via the internal jugular line, normal saline replacement via the peripheral venous line, and blood sampling via the arterial line.

The study period was divided into three 10-min intervals. Within each 10-min interval, in group H patients 7 $ml\ kg^{-1}$ of blood was removed and simultaneously replaced with 14 $ml\ kg^{-1}$ normal saline; Group C patients were undisturbed. The volume of saline infused was chosen to maintain normovolaemia according to the nomograms developed by Drobin and colleagues.[10] In both groups, blood samples for TEG and other measurements were taken at the end of each 10-min interval. At the end of the three 10-min intervals, Group H patients had had approximately 30% of their blood volume removed and replaced with normal saline. Three sets of blood samples for analysis were collected from each patient. If blood removal was completed within 10 min in a Group H patient, the patient remained undisturbed until the end of the 10-min interval, when blood samples were taken.

Blood samples were taken using a double syringe technique; the first 10 ml collected was discarded. Each sample was then sent for the following analysis: (i) immediately for haemoglobin (Hb) on a haemoglobin photometer (Hemocue, Hemocue AB, Angelholm Sweden); (ii) within 3 min for measurement by TEG (Haemoscope Corporation, Skokie, IL, USA) as fresh whole blood without celite. TEG parameters recorded included r-time, k-time, maximum amplitude (MA) and α. (iii) Stored in 0.109M trisodium citrate tubes (Vacuette, Greiner Labortechnik, Germany), 9 parts to 1 by volume for prothrombin time (PT), INR, activated partial thromboplastin time (aPTT), fibrinogen, protein C, AT III and thrombin-antithrombin complex. (iv) Stored in EDTA tubes (Vacuette, Greiner Labortechnik, Germany) for Hb, platelet count (PLT) and white cell count (WCC).

Complete blood counts were performed on an automated cell counter (Gen-S, Beckman-Coulter, USA). PT and aPTT were measured immediately after collection of blood samples and centrifugation at 3000 r.p.m for 10 min on an automated coagulometer (MDA-180, Organon-Technika, USA). Fibrinogen level was assayed by the Clauss method using a semi-automated coagulometer (Cobas Fibro, Roche, Switzerland). Tests for other coagulation parameters were batch processed, and plasma samples were stored at –70°C. Assays for protein C and AT III were done by chromogenic assays according to the manufacturer's instructions using commercial kits (Stachrom protein C and Stachrom AT III, both from Diagnostic Stago, France) on an automated coagulometer (ACL 3000™, Instrumentation Laboratory, Italy). Thrombin–antithrombin assay was performed by sandwich enzyme immunoassay using a commercial kit (Enzygnost TAT, Behring, Germany) according to the manufacturer's instructions.

Surgery was started after completion of blood sampling. More normal saline was then administered in Group H patients to keep the central venous pressure above 5 mm Hg, and autologous blood collection was performed in Group C patients.

Table 1 Patient characteristics. Data are mean (SD) or actual numbers

	Group H (n=10)	Group C (n=10)
Age (yr)	58.0 (43–70)	49.0 (19–65)
Body mass index (kg m^{-2})	20.6 (3.99)	21.8 (3.74)
Sex ratio (M/F)	5/5	8/2
Smoking (Y/N)	1/9	2/8
Diagnosis		
Hepatocellular carcinoma	7	7
Cholangiocarcinoma	1	1
Retroperitoneal tumour	–	1
Metastatic liver tumour	1	–
Carcinoma of pancreas	–	1
Carcinoma of gallbladder	1	–

Statistics

Statistical analysis was performed using the software programme Statistica release 4.5 (StatSoft, Tulsa, OK, USA). Intergroup comparisons of the changes in TEG parameters from control at each time point were performed using the Mann–Whitney *U* test. Intergroup comparisons of other coagulation tests and factors were performed using the unpaired two-tailed Student's *t* test. Changes in TEG parameters and coagulation tests over time were compared with the preoperative value in each group by repeated measures ANOVA. Where a statistically significant difference was detected, further paired comparisons were made between individual time points and the preoperative values using the Tukey HSD test. The significance level was set at $P<0.05$.

The sample size of ten in each group allowed us to detect with 80% power at this significance level a 30% difference in any parameter, assuming the standard deviation of the parameters to be approximately 25%. These approximations were estimated from previous similar studies.[4 8]

Results

A total of 20 patients were recruited. There were ten patients in each group. Patient characteristics and surgical details are given in Table 1.

Changes in r-time, k-time, MA and α are summarized in Figures 1–3. Group H had a significantly shorter r-time at 30 min (–30% *vs* +36% in control group C, $P<0.05$), a shorter k-time at 10, 20 and 30 min (–36% [Group H] *vs* +17% [GroupC], $P<0.01$; –37% *vs* +44%, $P<0.05$; and –45% *vs* +49%, $P<0.05$, respectively) and a wider α at 30 min (+71% *vs* +4%, $P<0.05$).

Changes in WCC, PLT, INR, aPTT, fibrinogen, thrombin–antithrombin complex, AT III and protein C are summarized in Table 2. Group H patients had a longer aPTT at 20 min (36.0 s [Group H] *vs* 31.3 s [Group C], $P<0.05$), lower fibrinogen at 30 min (2.1 g litre^{-1} *vs* 3.1 g litre^{-1}, $P<0.05$) and lower AT III at 30 min (0.64 IU ml^{-1} *vs* 0.90 IU ml^{-1}, $P<0.05$). There were no statistically significant

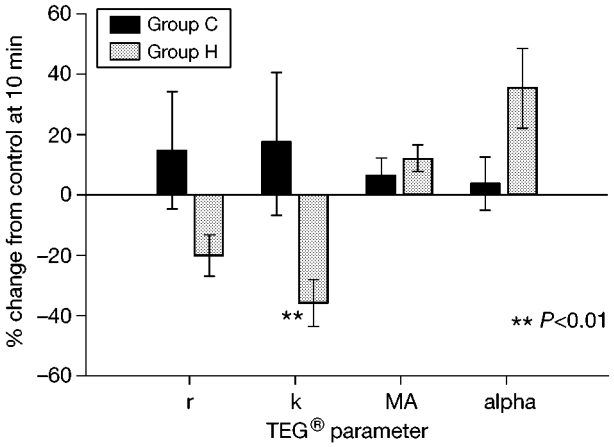

Fig 1 Comparison of TEG parameters (r-time, k-time, MA, α) between Group H and Group C at 10 min. **$P<0.01$ Group H *vs* Group C.

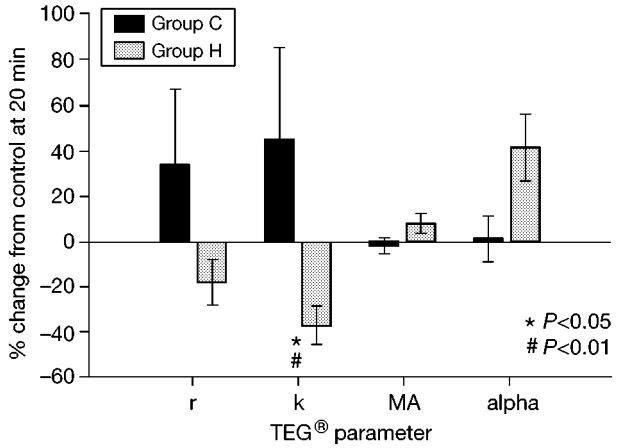

Fig 2 Comparison of TEG parameters (r-time, k-time, MA, α) between Group H and Group C at 20 min. * $P<0.05$ Group H *vs* Group C; #$P<0.01$ compared with time zero.

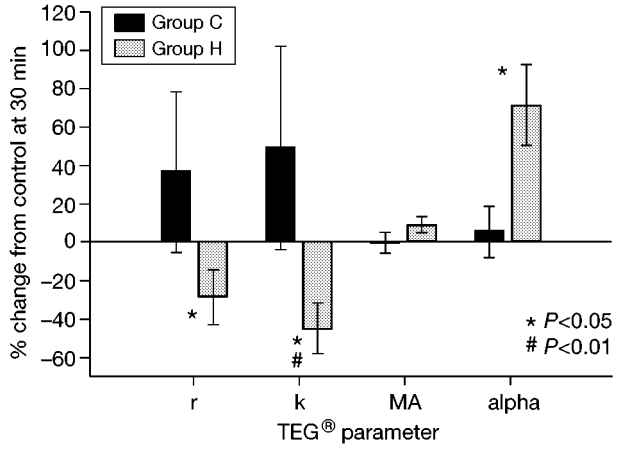

Fig 3 Comparison of TEG parameters (r-time, k-time, MA, α) between Group H and Group C at 30 min. * $P<0.05$ Group H *vs* Group C; #$P<0.01$ compared with time zero.

Table 2 Change of cellular and haemostatic parameters over time: Group H *vs* Group C. Data are mean (SD). **P*<0.05 Group H *vs* Group C (Student's *t*-test); †*P*<0.01; ‡*P*<0.001 compared with time zero (repeated measure ANOVA followed by the Tukey HSD test); PLT, platelet count; APTT, activated partial thromboplastin time; FIB, fibrinogen; AT III, antithrombin III; TAT, thrombin–antithrombin complex; PC, protein C; WCC, white cell count

		0 min	10 min	20 min	30 min
Group H (*n*=10)	PLT ($\times 10^9$ litre^{-1})	202 (75)	186 (71)	171 (61)‡	158 (59)‡
	INR	1.04 (0.12)	1.10 (0.098)	1.24 (0.288)	1.29 (0.080)
	APTT (s)	34.9 (4.5)	37.9 (6.6)	36.0 (2.8)*	42.2 (9.6)
	Fibrinogen (g litre^{-1})	3.18 (0.97)	2.70 (0.92)†	2.33 (0.79)‡	2.14 (0.67)*‡
	AT III (IU ml^{-1})	0.92 (0.26)	0.80 (0.23)‡	0.71 (0.22)‡	0.64 (0.20)*‡
	TAT (μg litre^{-1})	10.64 (6.88)	11.87 (8.10)	9.20 (3.81)	12.75 (7.41)
	PC (IU ml^{-1})	0.91 (0.24)	0.83 (0.24)	0.74 (0.23)‡	0.69 (0.19)‡
	WCC ($\times 10^9$ litre^{-1})	6.07 (2.39)	5.69 (2.48)	5.19 (2.13)‡	4.75 (1.90)‡
Group C (*n*=10)	PLT ($\times 10^9$ litre^{-1})	199 (78)	205 (81)	206 (81)	198 (72)
	INR	1.04 (0.06)	1.07 (0.07)	1.08 (0.07)	1.08 (0.08)
	APTT (s)	31.5 (4.0)	34.2 (8.6)	31.3 (4.8)*	36.5 (11.9)
	FIB (g litre^{-1})	3.29 (1.42)	3.17 (1.30)	3.17 (1.40)	3.07 (1.16)*
	AT III (IU ml^{-1})	0.96 (0.22)	0.90 (0.23)	0.89 (0.23)	0.90 (0.23)*
	TAT (μg litre^{-1})	22.03 (35.74)	26.69 (46.70)	12.44 (14.29)	15.09 (16.97)
	PC (IU ml^{-1})	0.85 (0.17)	0.82 (0.16)	0.80 (0.15) †	0.79(0.16)‡
	WCC ($\times 10^9$ litre^{-1})	6.67 (3.12)	6.43 (2.52)	6.60 (3.04)	6.40 (2.58)

differences in WCC, platelet count, INR, thrombin–antithrombin complex or protein C between Group H and Group C at any time point.

The changes in Hb over time in Group H and Group C are shown in Figure 4. There is steady haemodilution in Group H, with Hb values at 10, 20 and 30 min approximately 0.9, 0.8 and 0.7 times the Hb value at 0 min. Apart from thrombin–antithrombin complex, which became slightly elevated over time in group H (Table 2), all variables, including platelet count, WBC, fibrinogen, AT III and protein C, in Group H patients closely followed the decrease in Hb as a result of haemodilution (Fig. 5). There was a slightly larger drop in fibrinogen and AT III compared with Hb, but the difference was only significant statistically for fibrinogen at 10 min (P<0.05).

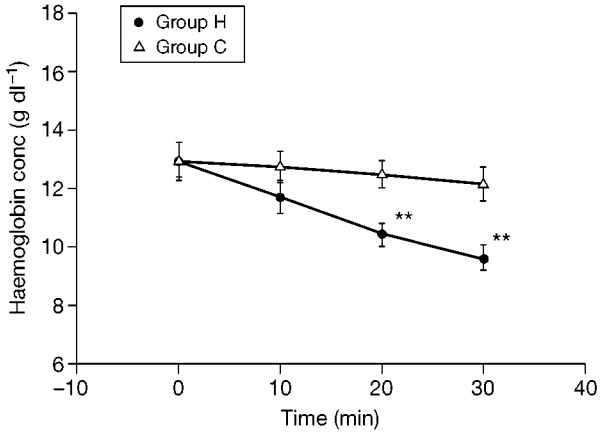

Fig 4 Change in haemoglobin concentration over time (***P*<0.01 compared with time zero).

Discussion

Our present study has confirmed the development of a hypercoagulability state with mild saline haemodilution *in vivo*. The effect is demonstrable using TEG as a shortened r-time and k-time and widened α at levels of haemodilution of 20–30%. These TEG changes were not found in the control group. We can therefore eliminate 'environmental factors' as the cause of these changes.

The degree of haemodilution in our study is more profound than in a previous similar study.[8] While we detected most of our TEG changes at 20% or more haemodilution, the previous study reported changes at only 10% haemodilution. It is therefore difficult to compare the magnitude of changes of the various TEG parameters in the two studies. However, the pattern of the changes observed is highly consistent in both studies. The most significant changes are observed in k-time and α, followed by changes in r-time and a modest change in MA. The effect of saline haemodilution on coagulation seems to be most significant in accelerating the rate of growth of clot strength.

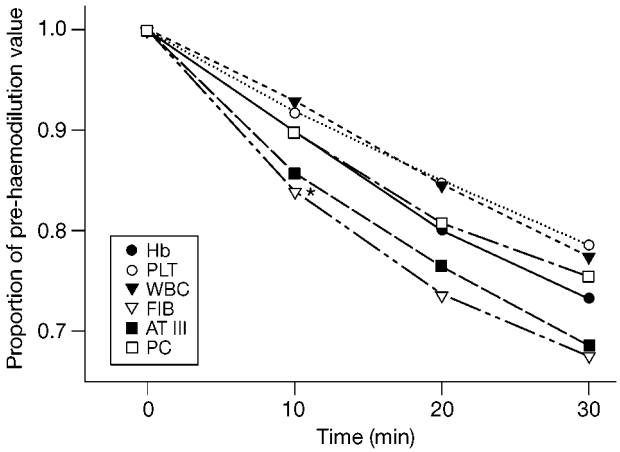

Fig 5 Proportional fall in various coagulation markers with respect to haemoglobin concentration in Group H (**P*<0.05).

Our study failed to identify the exact mechanism by which these changes occurred. A disproportionate reduction in concentrations of natural anticoagulants such as AT III with haemodilution has previously been suggested as a possible explanation.[8] We are unable to demonstrate this in our present study. The decrease in AT III and protein C followed closely that of Hb as haemodilution progressed and was paralleled by similar changes in fibrinogen concentration. Although in terms of absolute concentration no disproportionate dilution of AT III or protein C was observed, reduction in the concentration of anticoagulants could still be responsible for the hypercoagulability we and others have reported. In particular, the impact of AT III on the natural balance between coagulation and anticoagulation may be much higher than that suggested by its absolute concentration. AT III is the most potent thrombin inhibitor.[11 12] As thrombin plays a key role in several positive-feedback loops in the clotting mechanism, small changes in the concentration of AT III may have a profound effect on the initiation and amplification of the clotting process.[13]

Unlike other factors, the concentration of thrombin–antithrombin complex remained unchanged despite haemodilution in Group H. On the other hand, concentrations decreased with time in Group C (Table 2). This implies an increase in thrombin generation rate in Group H particularly as the concentration of AT III was progressively decreasing with haemodilution.

We have confirmed that acute haemodilution of 20–30% with normal saline *in vivo* induces a hypercoagulable state as measured by TEG in surgical patients under general anaesthesia. Our results confirm the findings of previous *in vivo* and *in vitro* studies. However, it is important to note that changes in haemostatic function will be very different with more profound haemodilution,[14–16] or haemodilution with other colloid solutions.[1 2 6 14 17–20] For example, administration of hydroxyethyl starch for haemodilution will impair platelet function through its effect on von Willebrand factor.[21 22]

The mechanism by which hypercoagulability occurs with mild haemodilution with saline remains intriguing. However, we have demonstrated the possibility of increased thrombin generation as a cause for this phenomenon, thrombin–antithrombin complex concentrations remaining unchanged despite haemodilution. Other mechanisms which have not been investigated in our study could also be responsible for this interesting phenomenon. For instance, thrombus formation on a collagen-reinforced thrombogenic device has been shown to be inversely correlated to haematocrit,[23] and platelet adhesion to an artificial perfusion chamber was found to correlate inversely with blood viscosity.[24] The conditions of these studies are very similar to those during TEG measurement, namely activation of platelets and coagulation while blood is in contact with a foreign surface. The pattern of TEG changes observed in our study and that of a similar previous study[8] with predominantly shortening of k-time and widening of α also suggest that facilitation of platelet interaction with platelet activating surfaces during haemodilution may be a possible mechanism. Further studies are required to define more clearly the mechanism and the significance of hypercoagulability induced by saline haemodilution. Although haemodilution has been advocated as the treatment of choice for preventing various thromboembolic diseases such as stroke, the results of this treatment modality have not been conclusive.[25] In a large prospective cohort study, a U-shaped relationship was found between haematocrit and the risk of stroke, with the risk of stroke increased in patients with higher or lower haematocrit levels.[26] The relationship between haemodilution and thrombosis and haemostasis is probably more complicated and heterogenous than we used to believe. For practical purposes, the development of hypercoagulability and its potential risks must be considered whenever crystalloid haemodilution to 10–30% is employed clinically.

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