The effect of hydroxyethyl starch (HES) on different fibrinogen quantification methods in elective neurosurgery.

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Påverkan av hydroxyetylstärkelse (HES) på olika fibrinogenbestämningsmetoder vid elektiv neurokirurgi.

Blodförlust och narkos kan orsaka minskad genomblödning av kroppens vävnader. För att motverka detta ges olika vätskor som dropp i blodbanan (infunderas) under operation. Fibrinogen – ett viktigt ämne för blodlevring – sjunker vid blödning och operation, dels genom att förbrukas men också genom utspädning med infusionsvätskor. En av dessa vätskor – hydroxyetylstärkelse – kan ge falskt höga nivåer av fibrinogen när mätning sker med s.k. optisk Clauss-metod, som är det vanligaste sättet att bestämma fibrinogen på, i laboratorier i Sverige och runtom i världen. Detta är av stor betydelse eftersom låga fibrinogenvärden vid blödning kan behandlas med koagulationsfaktorkoncentrat. Ett falskt högt fibrinogenvärde kan resultera i utebliven behandling. Effekten av hydroxyetylstärkelse på Clauss-metoden har bara studerats in vitro, d.v.s. när blodprov från frivilliga blandats med stärkelselösning i provrör. Hydroxyetylstärkelse används som rutin vid neurokirurgiska operationer och det finns inga studier på patienter som får stärkelse under operation (in vivo) och hur detta påverkar olika fibrinogenmetoder.

Syftet med vår prospektiva studie på neurokirurgiska patienter var därför att undersöka om hydroxyetylstärkelse orsakade förhöjda fibrinogenvärden mätt med optisk Clauss-metod. Av 30 neurokirurgiska tumörpatienter utvärderades 14 stycken som fått 1000ml stärkelseinfusion under operation, med konsekutiva blodprovstagningar. Fibrinogen mätt med två optiska Clauss-metoder och en immunologisk metod jämfördes. Den senare har enligt tidigare in vitro-studier inte påverkats av stärkelse. Även en viskoelastisk metod (ROTEM), som kan värdera fibrinstruktur/fibrinpolymerisering och bättre korrelera till blödning, studerades.

Resultaten av denna preliminära rapport av en större patientserie om totalt 40 patienter, påvisade en intraoperativ sänkning av fibrinogen efter stärkelseinfusion. Infusionerna orsakade inte falskt höga fibrinogenvärden mätt med de optiska Clauss-metoderna. Vid jämförelse av laboratoriemetoderna, skilde sig de två Clauss-metoderna åt. Den immunologiska metoden överensstämde med ROTEM, men bara preoperativ ROTEM-analys korrelerade till intraoperativ blödning.

The effect of hydroxyethyl starch (HES) on different fibrinogen quantification methods in elective neurosurgery.

Background: Fibrinogen, a plasma protein essential for clot formation, can reach critically low levels during bleeding. The most commonly used method to determine fibrinogen is the photometric Clauss method. Intravenous colloid fluids can lead to falsely high values of fibrinogen when measured with the photometric Clauss method in comparison to an immunologic method. The main objective of this study was to investigate the effect of hydroxyethyl starch colloid on three different fibrinogen determination methods during elective neurosurgery. A secondary aim was to compare rotational thromboelastometry, which assess clot structure, with the immunologic fibrinogen method and compare these methods with intraoperative blood loss.

Methods: Blood was collected from arterial catheters prior to surgery, after 1000 ml of starch, at end of surgery, and 3, 6 and 12 hours after surgery. A total of 30 patients scheduled for intracranial tumour extirpation were included after signed consent. Three different laboratory plasma fibrinogen quantification methods were assessed; two Clauss methods with photometric end-point determination using two different reagents (Multifibren U and Dade Thrombin) and one immunological assay (Zymutest Fibrinogen kit). Thromboelastometry was assessed with Maximum Clot Firmness.

Results: 14 of 30 patients who received 1000ml starch were statistically evaluated in this preliminary report. Plasma fibrinogen concentrations, measured with the photometric Clauss method (Dade Thrombin reagent) were higher preoperatively as compared to the immunologic measured fibrinogen. Starch infusion did not increase this difference during or after surgery. Maximum Clot Firmness in the thromboelastometry correlated with the immunologic method (r=0.76); preoperatively only thromboelastometry predicted intraoperative bleeding (r=-0.37).

Conclusion: In this first report on in vivo hydroxyethyl starch infusion effects on different photometric plasma-fibrinogen methods, no effect of starch was seen as compared to an immunologic assay. These results do not corroborate previous in vitro studies. Thromboelastometry correlated to the immunologic fibrinogen method, but better predicted blood loss.

INTRODUCTION

Fibrinogen is an essential coagulation factor in human plasma (p-fibrinogen) and the first factor to become critically low in the bleeding patient. Synthetic colloid fluids are commonly used during elective surgery to replace initial blood loss, maintain blood pressure and tissue perfusion. Colloids affect haemostasis by many mechanisms and can increase bleeding[2]. It is therefore important to adequately measure p-fibrinogen.

There are various methods to record fibrinogen. Most commonly used in Sweden and worldwide is the Clauss method, where functional fibrinogen concentration is inversely proportional to coagulation time after diluted citrated plasma has been activated with an excess of thrombin[3]. To detect the fibrin clot end-point of the reaction, automated coagulometers adopt two different principles: mechanical or nephelometric/photometric[4]. Recent in vitro studies have shown that synthetic colloids (e.g. dextran and hydroxyethyl starch (HES)) in particular – may interfere with the photometric Clauss method of p-fibrinogen determination[5-8], resulting in a significant overestimation in the range of 10-40 percent. Modifications of the method, developed for low concentration measurement, can overestimate fibrinogen by 50-120 percent. This may lead to inaccurate haemostatic management in critical bleeding and delay intravenous fibrinogen concentrate administration.

HES also affect fibrin polymerisation which can lead to decreased clot strength and increased bleeding. The strength and stability can be measured by point of care (POC) techniques (e.g. thromboelastography (TEG) and thromboelastometry (ROTEM)) which employs the viscoelastic properties of whole blood. These techniques are under ongoing evaluation in anaesthesiology and have been used sparsely to assess coagulation in neurosurgical populations[9-11].

The aim of this study was to assess different p-fibrinogen quantification methods in elective neurosurgical patients after routine infusions of low molecular weight (130/0.4) HES. As a secondary objective, thromboelastometry was compared with an immunologic method and correlated to intraoperative blood loss.

MATERIALS AND METHODS

This was a clinical screening study and ethical approval was obtained from the Regional ethical review board (Lund, Protocol DNR 2012/43). The operation schedule software 'Orbit' was used to plan and select patients up to two weeks in advance. Informed and signed consent was received from 30 consecutive patients admitted to the University hospital of Lund, Sweden for elective neurosurgery between January-May of 2012. Only adult (>18 of age) patients undergoing intracranial tumour resection and craniotomy were included. Patients with prolonged APTT and/or PT, low platelet count (<100), known congenital or acquired coagulation disorder or patients treated with coagulation- and/or platelet inhibitors were excluded from the study.

HES

The synthetic colloid most frequently used during intracranial procedures with tumour removal is Venofundin® 60 mg/ml (6% hydroxyethyl starch (MW 130kDa, substitution 0.42) in saline solution (Braun)). This colloid is used for replacing initial blood loss and for maintaining adequate blood pressure and tissue perfusion. In this study there was no intervention by the research staff in the HES infusion protocol. This was entirely handled by the anaesthetic personnel in charge of the patient.

Samples

Arterial blood samples were drawn from indwelling radial arterial catheters with a continuous flush system. A total of 43.2ml blood was collected in 2 citrated tubes (BD Vacutainer® 4.5ml 0.129M for plasma analysis and 2.7ml 0.109M for ROTEM analysis). Sampling was performed consecutively; before surgery (bfs), after 1000ml (1L) of HES infusion, at end of surgery (eos), 3 hours (3h) after surgery, 6 hours (6h) after surgery and 12 hours (12h) after surgery (in the morning of the first postoperative day).

After sampling, the 2.7ml tubes were placed in a heating block with a temperature of 37º C for a minimum of 30 minutes before ROTEM analysis. The 4.7ml citrated samples were centrifuged for 20 minutes at 2000 rpm to obtain the plasma fractions and then stored in a -85ºC freezer.

ELISA fibrinogen

Immunological measurement of p-fibrinogen was conducted with the Zymutest Fibrinogen kit (Hyphen BioMed) which is a two site immuno-assay, designed with rabbit polyclonal antibodies, specific for fibrinogen.

Photometric Clauss

A thrombin reagent is added in excess to a sample, ensuring the coagulation time is independent of thrombin. The fibrinogen concentration is then determined by means of a calibration curve. Clot detection is measured as a change in turbidity of the sample under a given period of time. Two reagents were used: Multifibren U (Dade Behring) and Dade Thrombin (Siemens). The assays were performed on a Sysmex 7000 automated coagulation analyzer.

Point of care Thromboelastometry (ROTEM)

We used rotational thromboelastometry (ROTEM® from Pentapharm Munich, Germany) to measure coagulation parameters in the samples. The device was handled in accordance to the manufacturer's recommendations. Two tests were performed: Extem® and Fibtem® (Pentapharm). In Extem the sample is activated with tissue factor to asses clot formation, fibrin polymerisation and fibrinolysis via the extrinsic pathway. In Fibtem the sample is also activated with tissue factor but included in the reagent is a platelet inhibitor (cytochalasin D). The parameters measured for Extem was CT (Clotting Time), CFT (Clotting Formation Time), AA (α-angle) and MCF (Maximum Clot Firmness). CT measures the initiation, CFT and AA the propagation of coagulation and MCF the strength of the final clot. In Fibtem we measured MCF which gives a qualitative assessment of functional fibrinogen and fibrin stability. Reference range used for MCF was 50-72mm (Extem) and 9-25mm (Fibtem).

Statistical Analysis

Statistical analysis was performed on SPSS v20 and Excel 2010. The data series were tested for normality with Kolmogorov-Smirnov and presented as box plots. Paired t-tests were performed to determine p-values. Significant differences were defined at a $p<0.05$ level.

We investigated levels of agreement between methods with Bland-Altman analysis[12]. In these diagrams, fibrinogen concentration is presented as mean values (x-axis). The difference between the two methods is depicted on the y-axis. Also shown in the diagrams are 'limits of

agreement' (upper and lower dotted lines) and mean difference – bias (central dotted line). If the differences are normally distributed, 95 per cent of the values lie within 'limits of agreement'. When there is high level of agreement between two laboratory methods, the differences is close to zero.

RESULTS

Twelve samples for optical measurement with the Dade Thrombin reagent were discarded due to technical errors during analysis – but will be reanalysed from extra frozen plasma vials, as will samples from another ten patients completed after the initial interim thirty patients analysis reported in this article. Four samples were not obtainable in the morning of the first postoperative day (12h) because of malfunctioning arterial catheters.

Table 1 depicts the demographic data of the patients. During the study period a total of nine patients received 500ml HES and fifteen patients received 1000ml. Four patients received 750ml, 800ml, 1500ml and 2000ml respectively. Three patients received no HES.

In this preliminary report only fourteen patients receiving 1000 ml HES were statistically evaluated.

Mean values of p-fibrinogen from the different assays are shown in figure 1. Values from before surgery indicate an intergroup difference. The photometric method with Multifibren U reagent indicates an agreement with ELISA at 1000ml and 12h postoperatively. Mean fibrinogen values measured with the Dade Thrombin reagent in all six sampling events are higher as compared to both ELISA and the photometric method with Multifibren U reagent.

In figure 2, box plots of the three different p-fibrinogen methods are presented. Photometric measurement with Multifibren U reagent showed no differences ($p=0.07$) as compared to ELISA. The photometric method with Dade Thrombin was different ($p=0.04$) from ELISA at all sampling events. The two photometric methods also showed intergroup differences ($p=0.0000002$) at all sampling events.

Bland-Altman analyses of ELISA versus photometric Clauss with Multifibren U reagent (figure 3) showed that preoperative ELISA is higher and then decreases. The two methods had a high level of agreement at 6h postoperatively.

Bland-Altman analyses of ELISA versus photometric Clauss with Dade Thrombin (figure 3) showed that ELISA is lower preoperatively, at eos and lowest at 6h. Consequently, Dade Thrombin fibrinogen is higher.

Figure 4 shows box plots of ROTEM-MCF with Fibtem and Extem tests. There was a ($p<0.05$) decrease in MCF from before to end of surgery.

Figure 5 verify a strong correlation between Fibtem-MCF and ELISA-fibrinogen in samples taken prior to surgery and at eos. Result from 6h after surgery, showed a moderate correlation.

In figure 6, scatter plots between bleeding and p-fibrinogen as measured by ELISA before surgery (27 patients) and bleeding vs. Fibtem-MCF before surgery (29 patients) showed no correlation between ELISA and blood loss. Bleeding vs. Fibtem-MCF showed a moderate negative correlation ($r=-0.37$).

DISCUSSION

Synthetic colloids are often administered to patients in the emergency room, intensive care unit (ICU) and surgical setting who are at risk of bleeding. HES is known for its hypocoagulative effect on haemostasis, with decreased levels of coagulation factor VIII (FVIII) and von Willebrand factor, compromised fibrin polymerization and impairment of platelet function[13,14]. Clinical bleeding complications have been reported for HES 470/0.7 and HES 200/0.5[15]. The introduction of third generation HES 130/0.4 was associated with a higher degree of safety and lesser effect on coagulation than its predecessors. This approach is now being questioned. In patients with severe head injury there were no differences in using HES 130/0.4 compared to HES 200/0.5 in mortality, renal function, bleeding complications and use of blood products[16,17]. In cardiovascular surgery there were also no advantages using this HES formulation[18].

In an in vitro study by Adam et al.[6] six different methods for p-fibrinogen measurement in HES-diluted blood were evaluated on the same automated coagulation analyzer. Five of the assays used photometric Clauss with Multifibren U and Dade Behring reagents. When HES was added to the samples (30% and 50% dilution rate) there was a significant overestimation of the concentration as reported by the device. An even more extreme overestimation took place when using a modification of the Clauss (with Multifibren U) method (by changing the ratio between plasma sample and reagent) to measure fibrinogen concentrations in the lower range (0.35-3.1 g/L). This modification was done to achieve more accurate results and higher precision of the measurement.

We did not use modifications of reagent/blood sample volumes in our study. Instead the calibration curve of the optical instruments was expanded down to 0.2 g/L to improve the sensitivity and specificity of fibrinogen measurements in the lower range (0.2-3.1 g/L).

The optical method with Dade Thrombin reagent significantly differed from the ELISA in the present study, with repeated perioperative blood samples taken from patients before surgery, immediately after in vivo infusion of 1000 ml HES, end of surgery and at 3, 6 and 12h after end of surgery. Figure 1 show mean fibrinogen values from the three different methods at the different sampling points, illustrating that there are no effects of HES, i.e. giving false positive fibrinogen levels after 1000ml infusions. Bland-Altman analyses indicated that the photometric Clauss method with Multifibren U was lower and with Dade Thrombin higher. But there is no evident HES effect on the differences at eos and at 6h as compared to preoperative values. More blood samples from our study needs to be analysed to support this result, not corroborating the results from previous in vitro studies[5-7].

ROTEM is an alternative method to measure fibrinogen concentration and also functional fibrinogen/fibrin in the clot structure. Clot structure depends on fibrin polymerization, how tight woven the fibrin strands are, the width of the fibrin threads, the lysability of the fibrin and interactions between platelets and the fibrin mesh. The latter is best indicated by Extem-MCF in the ROTEM assay.

Fibtem-MCF, i.e. Maximum Clot Firmness, when platelet function has been blocked, is generally regarded as the “best” parameter on fibrinogen concentration and fibrin clot

structure. It showed a significant strong correlation with ELISA preoperatively, but this correlation decreased at eos and at 6h (figure 5).

There was a significant decrease ($p<0.05$) in Fibtem-MCF and Extem-MCF from before surgery to eos. The patients started to recover already after 3h (figure 4). Some of our patients had a very low Fibtem-MCF after 1000ml and at end of surgery but normalized postoperatively until 12h. None of our patients needed reoperation for evacuation of postoperative hematoma. Other patients had a stronger coagulation activity as measured by the ROTEM at 12h postoperatively (figure 4) as compared to preoperative values, probably reflecting the acute phase response of fibrinogen and vanished effects of HES. This was corroborated by the higher postoperative plasma-fibrinogen concentrations at 12h as measured by the two photometric Clauss methods and the ELISA method (figure 2). HES may have a thromboprophylactic effect, but it has never before been addressed in neurosurgery[19,20]. Risks for arterial and venous thromboses are a concern also in neurosurgery[21]. At our hospital (SUS, Skane University Hospital) only mechanical calf compressions and elastic stockings are used for thromboprohylaxis in this setting due to concern for increased bleeding.

The administration of fibrinogen concentrate for hemostatic stabilization has been evaluated in several studies[22-25]. Often ROTEM is used to guide the therapy and assess fibrin polymerization and clot strength. In a recent study by Solomon et al.[26] concerns were raised when fibrinogen concentrate treatment decreased the correlation between different fibrinogen methods and Fibtem-MCF.

Current guidelines recommend treatment of a ROTEM-Fibtem-MCF <3mm, aiming at increasing Fibtem >8mm in trauma and in massive bleeding[25,27]. Administration of a coagulation factor fibrinogen concentrate after the end of cardiopulmonary bypass in elective cardiac surgery reduced bleeding and postoperative drainage losses[28]. This has also been verified in radical cystectomy[29]. No such studies have been performed in elective neurosurgery. There was a moderate negative correlation ($r=-0.37$) between preoperative Fibtem-MCF and total intra-operative blood loss in the present study. Maybe it is better to improve preoperative Fibtem-MCF than a specific fibrinogen plasma level with a preoperative fibrinogen concentrate – not studied in elective brain tumour surgery before.

Two studies, one experimental[30] and one patient study on congenital fibrinogen deficiency[31], indicate no increased risk for thromboses, but clearly also this matter needs to be addressed in prospective studies involving fibrinogen concentrate treatments[32]. Previous studies with recombinant FVIIa and prothrombin complex concentrates indicate a thrombosis risk with coagulation factor therapy[33,34].

Differences exist between general surgery and brain tumour surgery. Neurosurgical patients are at higher risk of developing a postoperative hypercoagulative state with complications as deep vein thrombosis (DVT) and pulmonary embolism (PE)[35,36]. Goobie et al.[37] showed that thromboelastography (TEG) can be used to trace hypercoagulation after intracranial procedures in pediatric neurosurgical patients. The suggested mechanism is that tissue thromboplastin, with high intracerebral density, is released into the circulation, subsequently initiating the coagulation cascade. Fibrinogen concentrate treatment has also been used in craniosynostosis surgery[38], where ROTEM-detected dilutional coagulopathy was reversed – no discussion on hypercoagulation was addressed. In a study by Goh et al[39], the Prothrombin (PT) and APPT values showed no significant changes implicating TEG as a better way of assessing coagulation.

Two patients were identified with severely low Fibtem-MCF (<3mm) after 1000ml HES. This raised an ethical issue; was the infusion protocol to be continued (with possible additional units of HES) although it could further impair coagulation? The need for coagulation factor concentrate therapy was assessed individually and our study never intervened in current neurosurgical routines.

There are some weaknesses and limitations to the present study. First, the patient group studied is small. Secondly, the intracranial tumours are different with highly varying constitution, vascular composition and surgical accessibility. This may affect bleeding and needs to be considered when comparing different coagulation methods to bleeding during surgery.

In conclusion, fibrinogen as measured by the photometric Clauss method may depend on what reagent is used. Our data do not support previous in vitro studies indicating that HES interact with the photometric Clauss method, giving falsely high fibrinogen levels as compared to ELISA assays. ROTEM correlated with the ELISA fibrinogen method preoperatively, but

after HES dilution this correlation deteriorated. Preoperative fibrinogen ELISA levels did not correlate to intraoperative blood loss, whereas ROTEM-Fibtem-MCF did. Further studies in elective neurosurgery need to address differences in fibrinogen assays, preoperative treatment with fibrinogen concentrate, thromboprohylactic effects of HES and individualized HES dosage to avoid dilutional coagulopathy.

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LEGENDS TO FIGURES AND TABLES

Table 1. Demographic data. Number of patients included in the study, gender, age, BMI (26 patients), infused volumes of HES and mean preoperative B-Hb (26 patients).

Figure 1. Three methods of p-fibrinogen quantification. Mean values (14 patients) of fibrinogen concentration as measured by photometric Clauss method with Multifibren U and Dade Thrombin reagents and ELISA in patients with 1000ml HES infusion.

Figure 2. Multifibren U, Dade Thrombin and ELISA. Box plots (14 patients) of fibrinogen concentrations from photometric measurements with Multifibren U (**a**), Dade Thrombin (**b**) and ELISA (**c**) in patients with 1000ml HES infusion. Photometric measurement with Multifibren U reagent showed no differences ($p=0.07$) as compared to ELISA. The photometric method with Dade Thrombin was different ($p=0.04$) from ELISA at all sampling events. The two photometric methods also showed intergroup differences ($p=0.0000002$) at all sampling events.

Figure 3. Bland-Altman plots. Agreement between ELISA and photometric Clauss with Multifibren U and Dade Thrombin reagents in 14 patients with 1000ml HES infusion. Y-axis shows the differences between ELISA and the Clauss methods. X-axis shows the mean fibrinogen values (g/L) of the methods being compared. The upper and lower dotted lines are 'limits of agreement' and these are specified as average difference ± 1.96 standard deviation of the difference. The central dotted line is mean difference (bias). See text for result presentation.

Figure 4. Maximum Clot Firmness. Box plots of Maximum Clot Firmness (MCF) with ROTEM-Fibtem (**a**) and Extem (**b**) assays in 14 patients with 1000ml HES infusion.

Figure 5. Correlation. Scatter plots showing correlation between Fibtem-MCF and ELISA in 14 patients with 1000ml HES infusion at different times; before surgery, end of surgery and 6h postoperatively.

Figure 6. Correlation. Scatter plots showing correlation between bleeding and p-fibrinogen as measured by ELISA before surgery (27 patients) and bleeding vs Fibtem-MCF before surgery (29 patients).

Table 1.

Gender, n=30	17 females, 13 men
Mean age (SD and Range), n=30	57,7 years (12.3 and 46)
Mean BMI (SD and Range), n=26	27,0 (4.9 and 17.6)
HES volume (n of patients)	0ml (2) 500ml (9) 800ml (2) 1000ml (15) ≥1500ml (2)
Mean preop B-Hb (SD and Range), n=26	144,8 g/L (14.8 and 61)

Figure 1.

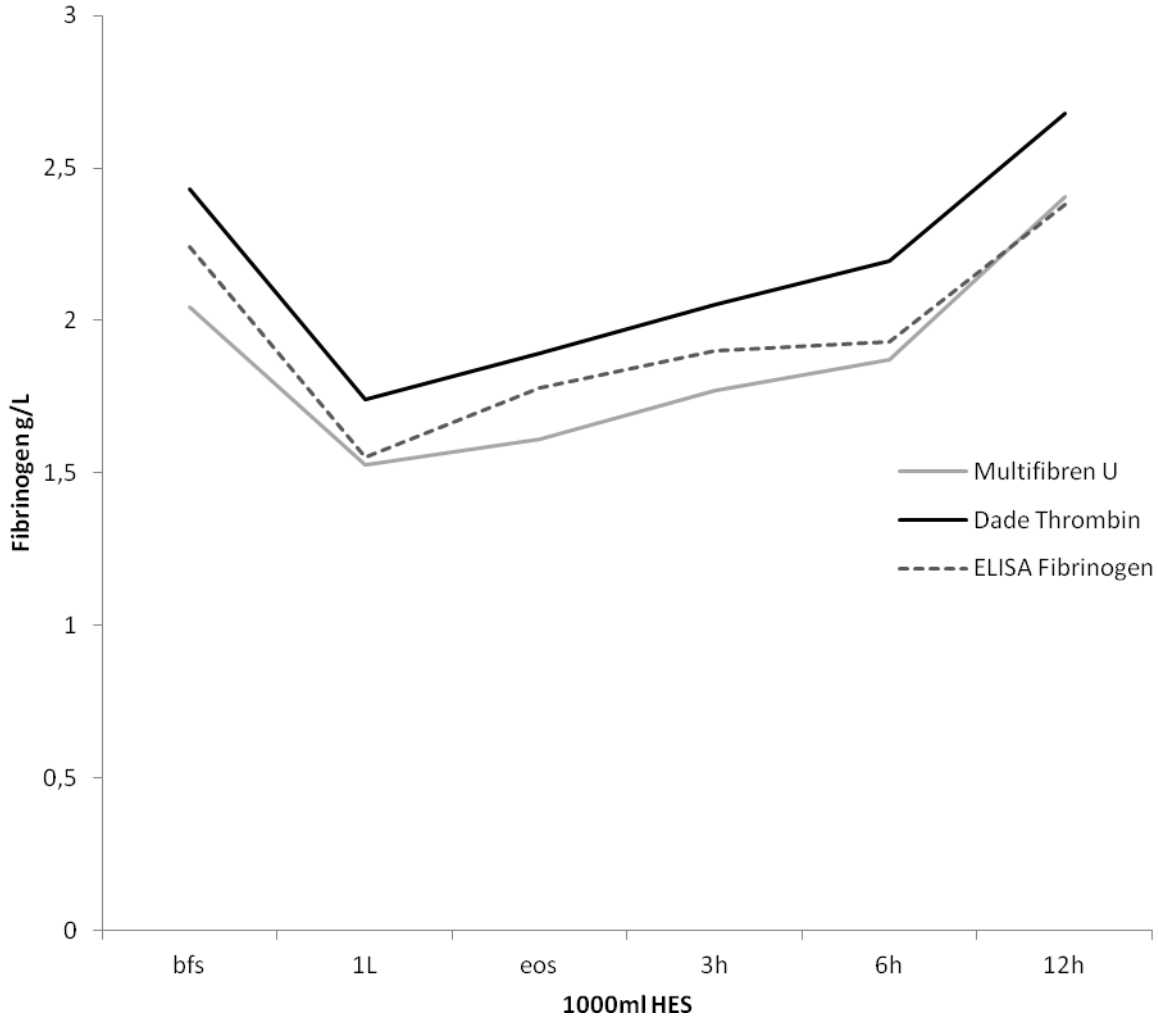
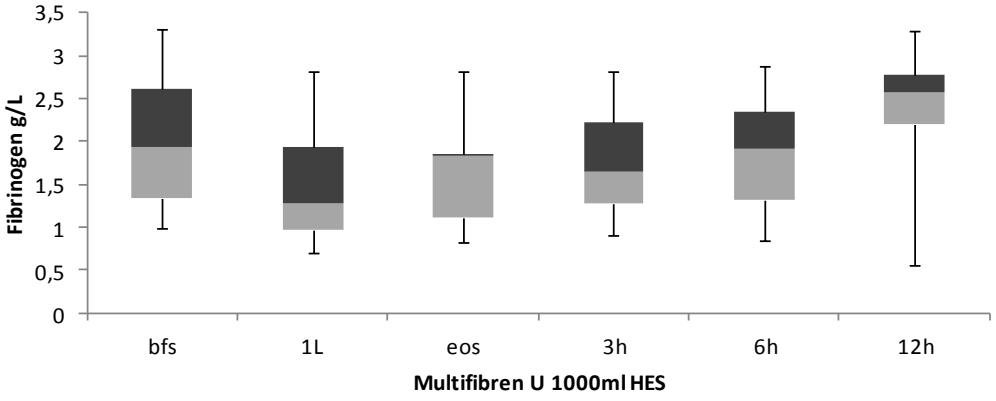
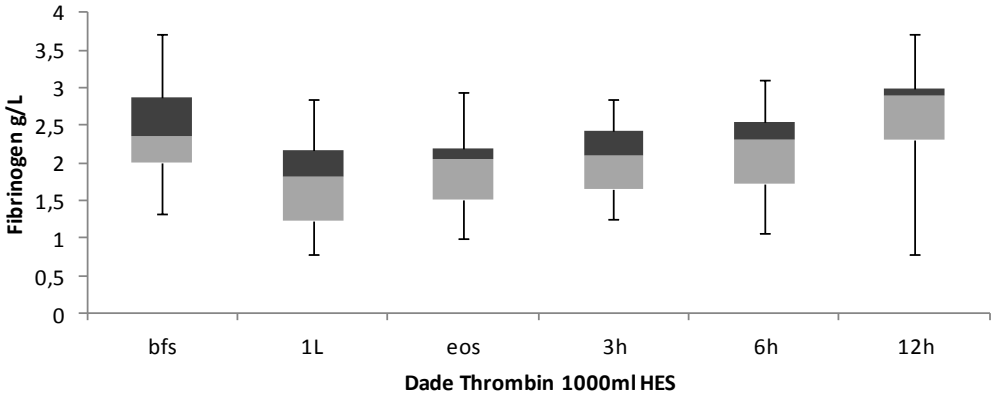


Figure 2 a-c.

a.



b.



c.

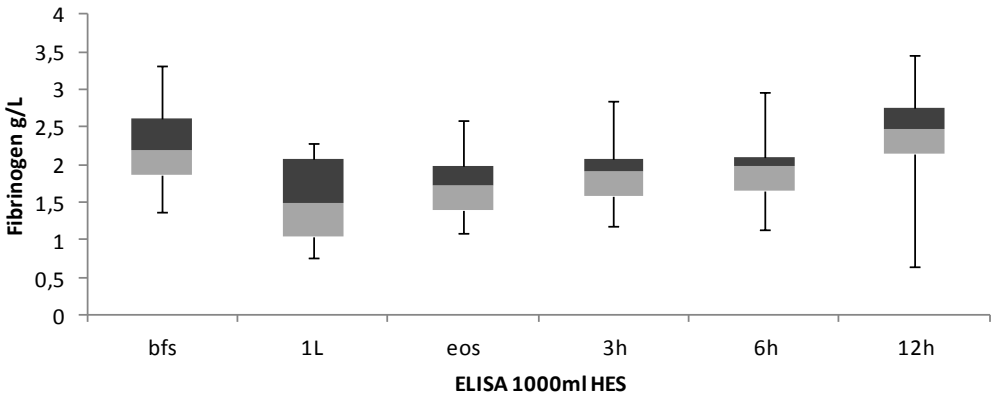


Figure 3.

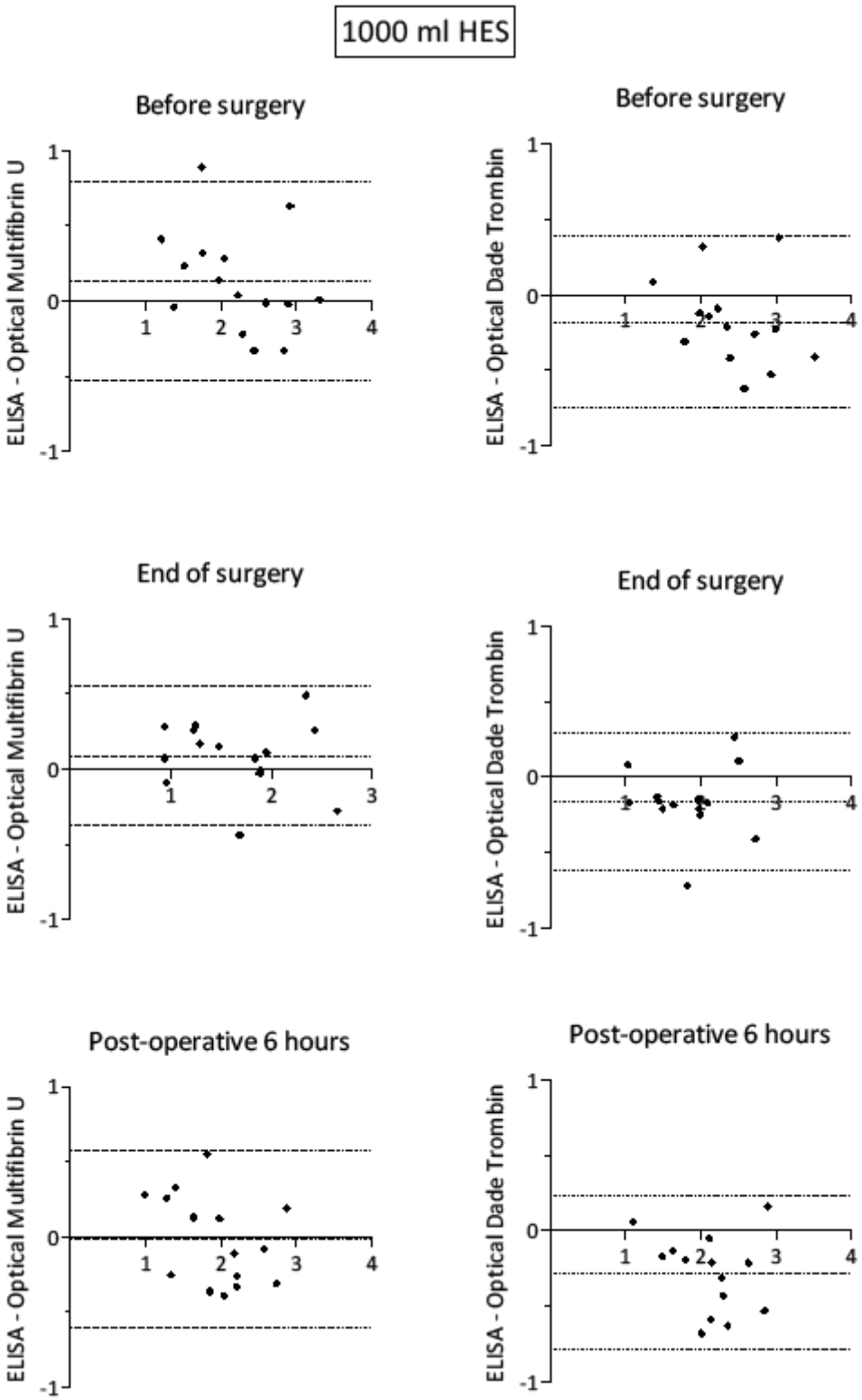
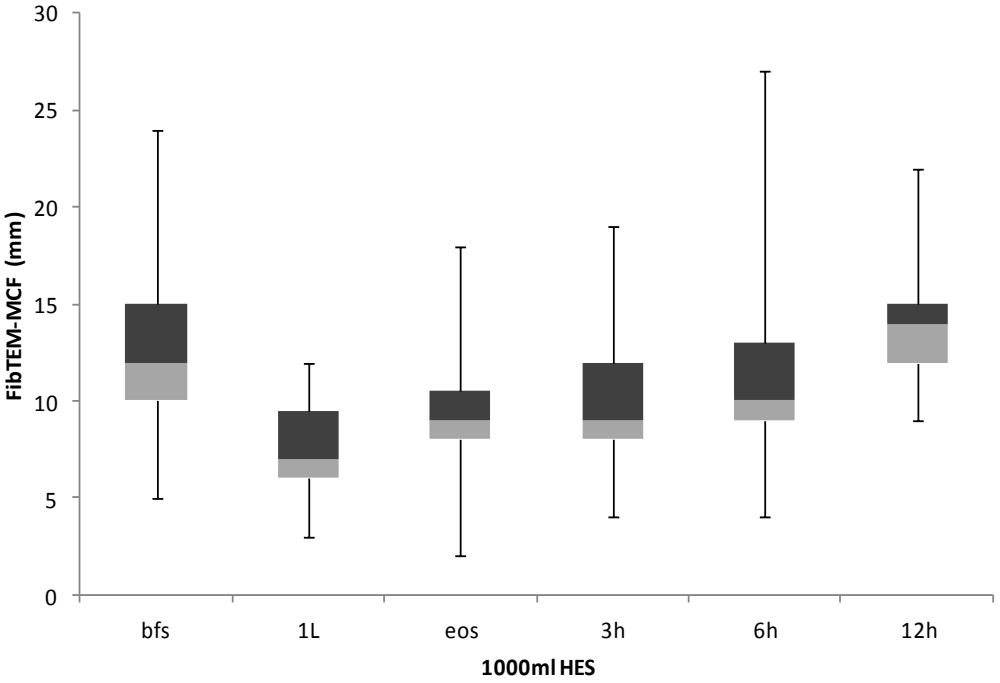

1000 ml HES
Before surgery
ELISA - Optical Multifibrin U
Before surgery
ELISA - Optical Dade Trombin
End of surgery
ELISA - Optical Multifibrin U
End of surgery
ELISA - Optical Dade Trombin
Post-operative 6 hours
ELISA - Optical Multifibrin U
Post-operative 6 hours
ELISA - Optical Dade Trombin

Figure 4 a-b.

a.



b.

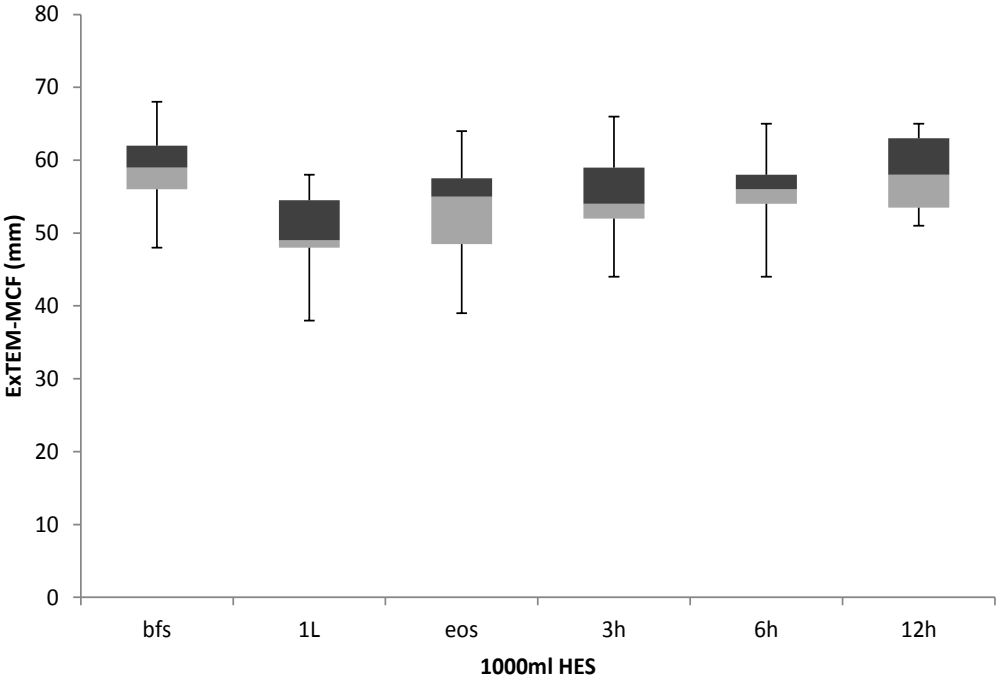


Figure 5.

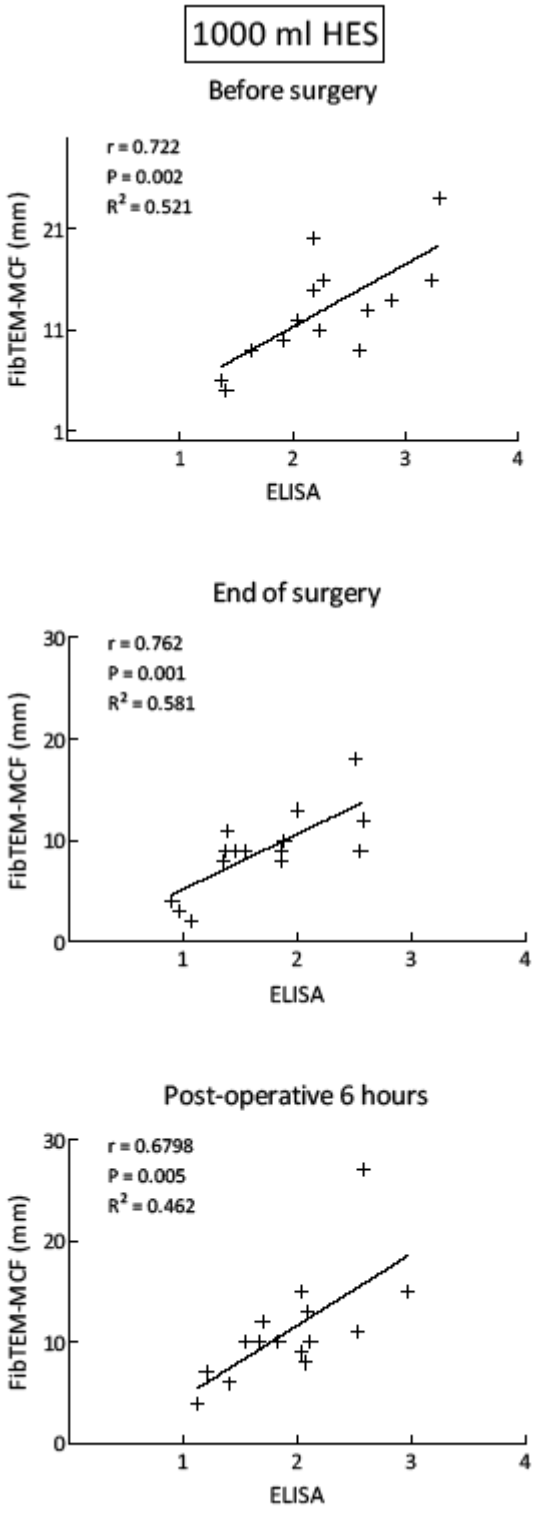
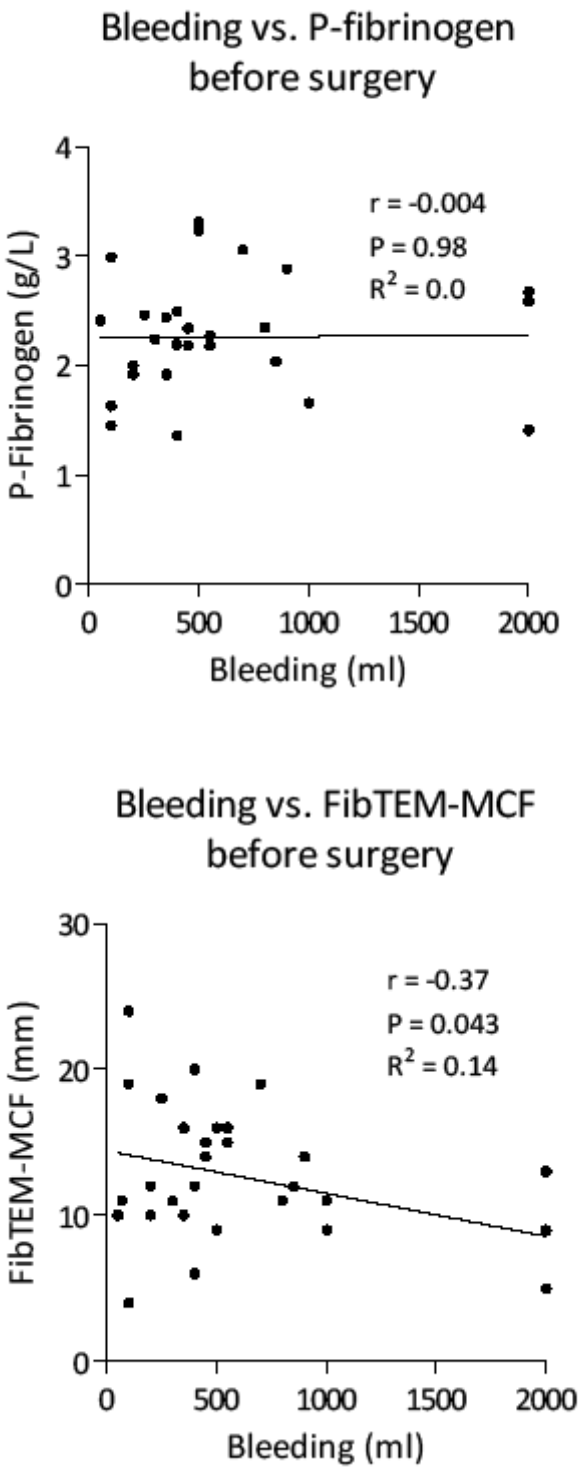

1000 ml HES
Before surgery
r = 0.722
P = 0.002
R² = 0.521
FibTEM-MCF (mm)
ELISA
End of surgery
r = 0.762
P = 0.001
R² = 0.581
FibTEM-MCF (mm)
ELISA
Post-operative 6 hours
r = 0.6798
P = 0.005
R² = 0.462
FibTEM-MCF (mm)
ELISA

Figure 6.


Bleeding vs. P-fibrinogen before surgery
P-Fibrinogen (g/L)
Bleeding (ml)
r = -0.004
P = 0.98
R2 = 0.0
0
1
2
3
4
0
500
1000
1500
2000
Bleeding vs. FibTEM-MCF before surgery
FibTEM-MCF (mm)
Bleeding (ml)
r = -0.37
P = 0.043
R2 = 0.14
0
10
20
30
0
500
1000
1500
2000