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Effects of balanced hydroxyethyl starch 6% (130/0.4) and albumin 5% on clot formation and glycocalyx shedding: Subgroup analysis of a prospective randomized trial

Tobias Kammerer[a,b,c,*], Nikolai Hulde[b], Eike Speck[a], Max Hübner[a,c], Alexander Crispin[d], Bernhard Zwissler[a], Peter Conzen[a], Vera von Dossow[b], Simon Thomas Schäfer[a,c], Klaus Hofmann-Kiefer[a,1], Markus Rehm[a,1]

[a] Department of Anaesthesiology, Hospital of the University, Ludwig-Maximilians-University Munich, Marchioninistr, 15, 81377 Munich, Germany
[b] Institute of Anesthesiology, Heart and Diabetes Center NRW, Ruhr University Bochum, Georgstr, 11, 32545 Bad Oeynhausen, Germany
[c] Walter-Brendel-Centre of Experimental Medicine, Ludwig-Maximilians-University Munich, Marchioninistr, 15, 81377 Munich, Germany
[d] Department of Medical Informatics, Biometry and Epidemiology, Ludwig-Maximilians-University Munich, Marchioninistr, 15, 81377 Munich, Germany

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ABSTRACT

Background: Intravenous fluids can impair coagulation and affect the endothelial glycocalyx, whereas glycocalyx shedding itself can cause an impairment of clot formation and firmness. We hypothesized that hydroxyethyl starch 6% (130/0.4) has a more distinct effect on coagulation and glycocalyx shedding than albumin 5%.

Methods: Presented data derive from an exploratory subgroup analysis of a prospective randomized, single-blinded trial comparing albumin 5% versus balanced hydroxyethyl starch 6% (130/0.4). Patients between 46 and 85 years undergoing cystectomy were included. Prothrombin time, plasma fibrinogen concentration, partial thromboplastin time, thrombelastometry and platelet function were analyzed before and after surgery. Glycocalyx components were assessed before and after surgery, 2 to 4 h after surgery and at 1st and 3rd post-operative day. Primary outcome parameter was the change of thrombelastometric variables at the end of surgery. Further variables included calculated blood loss, infusion amount and transfusion rate.

Results: 55 patients (albumin group $n = 28$; hydroxyethyl starch group $n = 27$) were included. Thrombelastometric variables were significantly more compromised in the hydroxyethyl starch than in the albumin group whereas platelet function, glycocalyx shedding, partial thromboplastin time, prothrombin time and fibrinogen were not different between groups. Mean intraoperative calculated blood loss was higher in the hydroxyethyl starch group (1557 ± 825 ml versus 1245 ± 709 ml; $p = 0.042$). Transfusion requirements did not differ.

Conclusion: Rotational thrombelastometric variables were significantly more reduced when hydroxyethyl starch was used compared to albumin 5%. This effect was independent from a shedding of the endothelial glycocalyx. However, results presented here are from a subgroup analysis and must be considered with caution.

Trial registration
EudraCT number 2010-018343-34.

1. Background

Volume replacement is an important tool to stabilize hemodynamic parameters and to ensure an adequate organ perfusion in perioperative patients. With colloidal solutions, this can be achieved significantly faster and more effectively than with crystalloids alone [1,2]. Unfortunately, colloid solutions can adversely affect coagulation and, in cases of a volume overload, lead to a degradation of the endothelial glycocalyx [3]. Besides, the amount of artificial colloids used in critical care patients is decreasing due to nephrotoxicity reported in previously published trials [4,5]. The effects of colloidal and crystalloid solutions on coagulation have been analyzed in various in vitro and in vivo studies [6].

The negative effects of colloids on coagulation do not only derive

* Corresponding author at: Georgstr, 11, 32545 Bad Oeynhausen, Germany.
E-mail address: Tobias.Kammerer@ruhr-uni-bochum.de (T. Kammerer).
[1] Equal contributors.

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from hemodilution alone but also from a direct impairment of platelet function and interaction with fibrin polymerization resulting in an impairment of clot firmness, especially when hydroxyethyl starches where used [7–9]. However, the influence on coagulation is not only seen with artificial colloids but also with albumin [10]. Albumin, like any other intravenous fluid, can generate hemodilution and decrease plasma fibrinogen concentrations. Additionally, surgical trauma and hypervolemia can cause a disruption of the endothelial glycocalyx which is a vulnerable structure on the luminal side of the vascular endothelium [3,11,12]. Glycocalyx shedding itself is associated with coagulopathy and fibrinolysis in trauma patients [13]. Rotational thrombelastometry is a quick and easy usable bedside point-of-care diagnostic to evaluate clot formation and stability [14]. While the negative influence of intravascular fluid administration on clot formation and firmness has been demonstrated in many cases, the impact of crystalloid and colloid solutions on platelet function is discussed controversially [15,16]. To date, there are no randomized clinical trials examining the differences between human albumin and hydroxyethyl starch in elective non-cardiothoracic surgery regarding thrombelastometric variables and platelet function.

Due to previous in-vitro and in-vivo studies we hypothesized that hydroxyethyl starch 6% (130/0.4) has a more distinct effect on thrombelastometric variables and glycocalyx shedding compared to albumin 5%. In an exploratory subgroup analysis of a prospective, interventional, randomized, single-blinded trial we therefore evaluated the effects of both colloid solutions on routine coagulation parameters, rotational thrombelastometry, platelet function and glycocalyx shedding in non-critically ill surgical patients. Additionally, we hypothesized that glycocalyx shedding itself might impair clot firmness and coagulation variables. Primary outcome parameter was a possible change of thrombelastometric variables until the end of surgery.

2. Materials and methods

The presented data derive from an exploratory subgroup analysis of a single-center, open-labelled, prospective, randomized, single-blinded trial with two parallel patient groups, comparing human albumin 5% and balanced hydroxyethyl starch 6% (130/0.4) to determine effects on acute changes in renal function. Ethical approvals were obtained from the Ethics Committee of the Ludwig-Maximilians-University of Munich (reference number: 311-11) and the responsible drug administration authority (Paul-Ehrlich-Institute of the German Federal Ministry of Health). The main study was registered at the European Medical Association (EudraCT number: 2010-018343-34; principal investigator: B.Z.; registration: July 26, 2011). For this subgroup analysis an amendment was written and approval from the Ethics Committee was given in March 2013. Written informed consent was obtained from all patients. Patients analyzed in this subgroup were recruited from April 2013 to February 2015 at the University Hospital Munich. The original trial protocol was published in 2015 [17]. Results concerning the primary endpoint and further variables were published in 2018 [18]. This manuscript adheres to the applicable CONSORT guidelines. Patients between 18 and 85 years undergoing surgical cystectomy were eligible for inclusion. After randomization, case history of preexisting coagulopathies was evaluated using a standardized questionnaire (see Supplemental Digital Content A). Patients with a preoperative therapeutic platelet inhibition or anticoagulant therapy within the last 7 days before surgery or clinical signs of a coagulopathy were excluded from this subgroup analysis. Medication with prophylactic doses of aspirin (maximum 100 mg/d) and/or low-molecular-weight heparin (enoxaparin; maximum 2×40 mg/d) until day before surgery was permitted. Further exclusion criteria were: Unfavorable prognosis (e.g. palliative surgical care in cases of obstruction of the efferent urinary tract); evidence for metastatic disease; known coagulopathy or platelet dysfunction; preoperative creatinine clearance < 30 ml/min, preoperative chemotherapy with a nephrotoxic drug (e.g. cisplatin), application of colloidal infusion solution within 24 h before surgery, history of hypersensitivity to one of the investigational drugs, abuse of drugs or alcohol; simultaneous participation in another clinical trial, pregnancy or nursing, and premenopausal women of childbearing potential without hormonal contraception for the entire study period.

Patients received general anesthesia (induction with propofol 2 mg/kg/BW, sufentanil 0.4 μg/kg/BW and rocuronium 0.6 mg/kg/BW followed by propofol 5 mg/kgBW/h or sevoflurane 0.8 to 1.0 minimum alveolar concentration (MAC) in combination with remifentanil 0.1 μg/kg/min) combined with a thoracic epidural anesthesia (TEA; epidural ropivacaine 0.2% in combination with epidural sufentanil). Intraoperative advanced hemodynamic monitoring was performed using the Vigileo© Monitor with FloTrack-Sensor© (Edwards Lifesciences Corporation, USA).

Standard coagulation variables (hemoglobin, hematocrit, platelets, prothrombin time (PT), activated partial thromboplastin time (aPTT), plasma fibrinogen concentrations (Clauss method)) were measured before colloid administration and immediately after surgery. To evaluate clot formation and firmness, rotational thrombelastometry (ROTEM© delta analyzer, Instrumentation Laboratory, Bedford, MA) was carried out before colloid administration and immediately after surgery. In detail, INTEM (screening test for the intrinsic hemostasis system), EXTEM (screening test for the extrinsic hemostasis system) and FIBTEM (EXTEM-based assay for the fibrin-dependent part of the clot) were used to determine clotting time (CT), clot formation time (CFT), maximum clot firmness (MCF), maximum clot elasticity (MCE) and clot amplitude after 10 (A10) and 20 (A20) minutes. Platelet function was examined at similar time points using impedance-aggregometry (Multiplate® analyzer, Roche Diagnostics GmbH, Unterhaching, Germany) with thrombin receptor activating peptide-6 (TRAP), adenosine diphosphate receptor (ADP) and arachidonacid (ASPI) stimulation and platelet function analyzer (PFA-100) with ADP and epinephrine. The use of impedance-aggregometry (Multiplate®) and platelet function analyzer (PFA-100) enables the detection of platelet dysfunctions and disorders of the primary hemostasis as well as the influence of platelet inhibitors with a high negative predictive value [19–21].

A potential shedding of the endothelial glycocalyx was verified by measuring serum hyaluronan, syndecan-1 and heparan sulfate concentrations. These glycosaminoglycans are major constituents of the endothelial glycocalyx and elevated serum levels of these glycosaminoglycans are an indicator for endothelial damage [12,13,22]. Normal serum values of circulating glycocalyx components in healthy volunteers were published by Hofmann-Kiefer et al. in 2013 for hyaluronan (median 86 ng/ml; interquartile range 82.1/93.6 ng/ml), syndecan-1 (median 66.4 ng/ml; interquartile range 50.7/90.7 ng/ml) and heparan sulfate (median 6200 ng/ml; interquartile range 5400/8900 ng/ml) [23]. Glycocalyx components were measured before colloid administration and after surgery, 2 to 4 h after surgery and at 1st and 3rd postoperative day according to the protocol of the main trial [17]. Blood samples were centrifuged for 10 min at 3000 rpm and serum was stored at -80 °C immediately after centrifugation. Enzyme-linked immunosorbent assay (ELISA) kits were used to analyze serum hyaluronan, syndecan-1 and heparan sulfate concentrations, and were performed according to the manufacturers' protocols (Diaclone SAS, Besançon, France for syndecan-1; Echelon Biosciences Inc., Salt Lake City, UT, USA for hyaluronic acid; Fa. Cusabio Art.Nr.: CSB-E09585h for heparan sulfate). The administration of infusions, vasoactive drugs and transfusions was done according to a goal directed protocol with different target variables depending on ASA classification and cardiovascular comorbidities, as published recently [18]. Fluid losses (urine, perspiratio insensibilis) were replaced by balanced crystalloid Ringer's acetate solution in a ratio of 1:1. Blood loss was replaced with either albumin 5% or balanced hydroxyethyl starch 6% (130/0.4) in a ratio of 1:1 up to a maximum dose of 30 ml/kg/d. This balanced hydroxyethyl starch solution is characterized by the addition of acetate and a reduced

chloride concentration and its modification is intended to decrease the risk of a hyperchloremic metabolic acidosis. Intraoperative blood loss was calculated using a modified formula previously published by our study group [2,24,25]. This formula uses repeated hematocrit measurements, body surface areas well as the amount of transfused red blood cells to calculate the loss of erythrocytes. In comparison to the original method, it is possible to dispense with the administration of indoncyanine green as an indicator under the assumption of normovolemia. The complete formula can be found in our previous publication [2].

2.1. Statistical analysis

The detailed statistical analysis and power calculation of the main trial are described in the protocol published in 2015 [17]. Outliers were evaluated, but no action was necessary. All randomized patients were included in the statistical analysis. The statistician was blinded to groups. In case of metrical variables, the Mann-Whitney *U* test was used, and Fisher's two-sided exact test for dichotomous ones, respectively. Simple and multiple linear regression analysis was used to adjust for confounders. A two-sided p-value of ≤ 0.05 was considered statistically significant. For repeated measurements of related groups, the Wilcoxon test and a two-way ANOVA with repeated measures and adjustment of the alpha level by using the Holm-Bonferroni method were used [26]. All analyses were performed using SPSS statistics version 23 for Windows (IBM Corporation, Armonk, NY, USA).

3. Results

After amendment and ethics approval 60 patients were screened for participation in this randomized subgroup analysis of which 6 had to be excluded (1 patient had exclusion criteria for the main trial, 2 patients had a history of coagulopathy evaluated by a standardized questionnaire and 2 withdrew consent). In total 55 patients were included in this subgroup analysis (albumin $n = 28$; hydroxyethyl starch $n = 27$). The participant flow diagram is shown in Fig. 1. Demographic data did not differ between both groups, except mean patient's age, which was higher in the hydroxyethyl starch group (mean values and standard deviation: 70 ± 9 years versus 65 ± 9 years; p = 0.021; see Table 1). Global coagulation variables were not different at baseline and changed significantly until end of surgery in both groups (see Table 2). Baseline variables of thrombelastometry and platelet function tests were comparable in both groups (see Supplemental Table 1). At end of surgery, absolute values of FIBTEM thrombelastometry were significant more compromised in the hydroxyethyl starch group whereas further thrombelastometric variables and platelet functions tests did not differ (see Supplemental Table 2). Considering the relative changes from beginning to end of surgery, statistically significant differences between both groups could be detected: using INTEM thrombelastometry, CFT (p = 0.021), A10 (p = 0.004), A20 (p = 0.002), MCF (0.005) and MCE (p = 0.006) were more compromised in the hydroxyethyl starch than in the albumin group (see Table 3). Comparable results were found in FIBTEM thrombelastometry for A10 (p = 0.001), A20 (p = 0.002), MCF (p = 0.001) and MCE (p = 0.001) (see Table 3). Using EXTEM thrombelastometry, MCF (p = 0.046) and MCE (p = 0.035) were more altered in the hydroxyethyl starch than in the albumin group (see Table 3). In all applied thrombelastometric tests, percentage change of clotting time was comparable in both groups (CT_{INTEM} p = 0.168; CT_{EXTEM} p = 0.570; CT_{FIBTEM} p = 0.980; see Table 3). Concerning platelet function, no significant intergroup differences were detected by Multiplate® or PFA-100 (see Table 3). In terms of glycocalyx shedding, no differences between both groups could be noticed until the 3rd postoperative day (see Table 4). In both groups serum hyaluronan showed an initial decrease from baseline until the end of surgery ($p < 0.001$) followed by an increase until the 3rd postoperative day ($p < 0.001$) whereas syndecan-1 increased immediately until 2 to 4 h after surgery only in the hydroxyethyl starch group (p = 0.037) (see Table 4). Concerning heparan sulfate no significant changes during the study period as well as no differences between groups were observed (see Table 4). Duration of surgical procedure was comparable in both groups (mean values and standard deviation: albumin group 217 ± 52 min versus hydroxyethyl starch group 226 ± 58 min; p = 0.501). The mean amount of crystalloids given until the third postoperative day was comparable in both groups (p = 0.409) whereas the average volume of perioperative colloid infusion until transfer from the operation theatre was higher in the hydroxyethyl starch group (mean values and standard deviation: hydroxyethyl starch group 1590 ± 640 ml versus albumin group 1260 ± 550 ml; p = 0.050) (see Table 5). Intraoperative transfusion rates for packed red cells (hydroxyethyl starch group 25.9% versus albumin group 25%; p = 0.999) and fresh frozen plasma (hydroxyethyl starch group 7.4% versus albumin group 7.1%; p = 0.999) did not differ. Calculated intraoperative blood loss was higher in the hydroxyethyl starch group (mean values and standard deviation: hydroxyethyl starch 1557 ± 825 ml versus albumin 1245 ± 709 ml; p = 0.042). To analyze confounding effects of age and further independent variables on blood loss, a linear regression was performed. Neither age alone ($R^2 < 0.001$; p = 0.898) nor combination of ASA classification, preoperative medication with anticoagulatory drugs and BMI ($R^2_{corrected} = 0.003$; p = 0.373) were correlated with intraoperative blood loss. Similar results were found for change of thrombelastometric variables (data not shown). Hemodynamic variables and need for vasopressors did not differ between both groups (data not shown). All infusion requirements and transfusion rates are given in Table 5.

4. Discussion

In this subgroup analysis of a single-center, prospective, randomized, single-blinded trial data from 55 elective surgical patients were analyzed. The investigation focused on coagulation, clot firmness, platelet function and glycocalyx shedding. In an elective surgical setting without critically ill patients, intraoperative blood loss was replaced with either balanced hydroxyethyl starch 6% (130/0.4) or albumin 5% in a ratio of 1:1. This procedure led to an impairment of coagulation variables in both groups, whereas clot firmness was significantly more reduced when using hydroxyethyl starch 6%. Additionally, blood loss was higher in the hydroxyethyl starch group. However, these results derive from an exploratory subgroup analysis and should therefore considered with caution.

Any intravenous fluid administration can provoke coagulopathy, due to dilutive effects [6]. This is even more relevant in case of colloidal solutions, which usually are applied only after considerable blood loss. Dilution itself can explain changes of global coagulation parameters observed in both groups of the current investigation. However, variables like prothrombin time are nonspecific and give no information about clot stability. We therefore used thrombelastometry and impedance aggregometry to detect coagulopathies and changes of clot firmness in our trial. Regarding variables of thrombelastometry, statistically significant differences were seen between both groups. The more pronounced effect of hydroxyethyl starch on thrombelastometric variables is in accordance with prior in-vitro and clinical investigations. Winstedt et al. compared the influence of colloidal solutions on clot formation, firmness and stability [10]. In this trial with healthy volunteers, hemodilution with different colloidal solutions followed by rotational thrombelastometry was investigated. The authors observed a lesser degree of coagulopathy when albumin was used compared to hydroxyethyl starch 6% (130/0.4). The same study group examined dilutional coagulopathy and its possible correction by fibrinogen and factor XIII in another in-vitro study in 2014 [27]. The authors compared the effects of hemodilution with hydroxyethyl starch 6% (130/0.4) and Ringer's acetate using rotational thrombelastometry. Again, they found a significant impairment of clot firmness in both groups. This effect was even more pronounced when hydroxyethyl starch was used. A possible

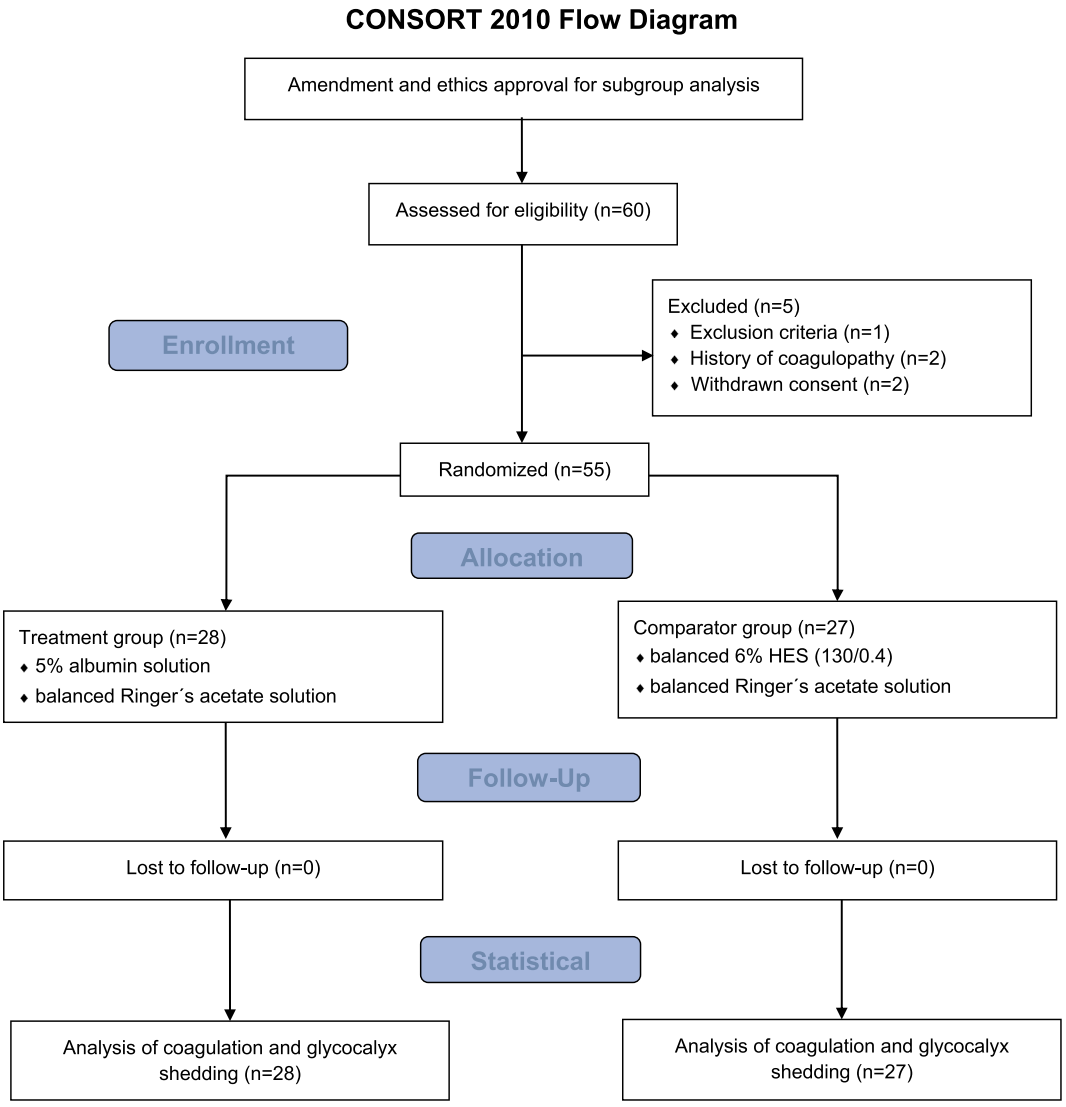

Fig. 1. Participant flow diagram.

Table 1
Demographic data.

	Albumin (n = 28)	HES (n = 27)	p-Value
Ileum conduit/neobladder, no.	12/16	15/12	0.423
Gender distribution, no. (%)			0.177
Female	8 (28.6)	3 (11.1)	
Male	20 (71.4)	24 (88.9)	
Age [y]	65 ± 9	70 ± 9	0.021
Height [m]	1.75 ± 0.1	1.74 ± 0.1	0.371
Body weight [kg]	84 ± 18	79 ± 13	0.328
Body mass index [$kg*m^{-2}$]	27.2 ± 5.3	26.1 ± 4.1	0.391
ASA classification, no. (%)			0.872
ASA 1	2 (7.1)	1 (3.7)	
ASA 2	9 (32.1)	11 (40.7)	
ASA 3	16 (57.1)	14 (51.9)	
ASA 4	1 (3.6)	1 (3.7)	
Preoperative medication*, no. (%)			0.238
Aspirin	4 (14.3)	8 (29.6)	
Enoxaparin	5 (17.9)	1 (3.7)	
Both	2 (7.1)	1 (3.7)	

Metric values given as mean ± SD. HES = Hydroxyethyl starch; ASA = American Society of Anesthesiologists. * = prophylactic non-therapeutic dosages. Mann-Whitney-*U* test for metrical variables; Fisher's exact test (two-sided) for dichotomous variables.

explanation for these findings could be an interaction of hydroxyethyl starches with fibrin network structure and, consecutively, an impairment of clot firmness [7]. The influence of colloidal solutions on clot stability can not only be detected ex-vivo, but also in clinical studies: The influence of different hydroxyethyl starch solutions on coagulation was investigated in patients undergoing total hip arthroplasty [28]. Shin et al. compared 10% pentastarch (260/0.45) with balanced and non-balanced hydroxyethyl starch 6% (130/0.4) and detected a reduced impairment of clot firmness when colloidal solutions with low molecular weight were administered. Comparable results were seen by Gandhi et al. in patients undergoing major orthopedic surgery. The authors measured nadir factor VIII and von Willebrand factor activity and found significantly lower levels when hydroxyethyl starch 670/0.75 was used compared to hydroxyethyl starch 130/0.4. Additionally, they observed a significantly higher amount of transfused erythrocytes in patients who needed erythrocyte transfusions [29]. The data presented here demonstrate a greater effect of hydroxyethyl starch solution on thrombelastometric variables compared to albumin. Although higher blood losses were calculated in the hydroxyethyl starch group, this does not suggest that changes of thrombelastometric parameters triggered this effect.

The influence of intravascular fluid therapy on platelet function is somewhat controversial. While some experimental studies show a

Table 2
Global coagulation parameters.

	Albumin (n = 28)	HES (n = 27)	p-Value
Baseline measurements			
Hemoglobin [g/dl]	12.2 (11.1/13.6)	12.5 (11.1/13.9)	0.775
Hematocrit	0.38 (0.34/0.40)	0.38 (0.34/0.42)	0.820
Platelets [G/l]	232 (186/282)	244 (215/321)	0.122
Prothrombin time [% activity]	90 (85/95)	90 (90/100)	0.377
Fibrinogen (Clauss method) [mg/dl]	305 (255/446)	309 (266/500)	0.597
Partial thromboplastin time [*sec*]	24 (22/27)	24 (22/26)	0.707
Percentage change from beginning to end of surgery			
Hemoglobin	−17.5 (−22.5/−11.9)*	−22.8 (−28.1/−14.6)*	0.042
Hematocrit	−17.1 (−22.5/−11.1)*	−23.3 (−27.9/−15.4)*	0.051
Platelets	−20.7 (−29.2/−9)*	−27 (−31.5/−17)*	0.154
Prothrombin time	−20 (−25/−11.4)*	−22.2 (−28.6/−11.8)*	0.498
Fibrinogen (Clauss method)	−28.2 (−34/−19.1)*	−27.8 (−36/−17.3)*	0.930
Partial thromboplastin time	+4.6 (± 0/+11.4)*	+12 (+4.7/+17.1)*	0.060

All values given as median (interquartile range). HES = hydroxyethyl starch. Wilcoxon signed-rank test for repeated measurements. Mann-Whitney-*U* test for metrical variables.

* Significant change from baseline ($p < 0.001$).

significant influence of hemodilution on platelet function, this finding could not be confirmed in other studies [15,16,30]. Accordingly, we found no significant intraoperative changes of platelet function and especially no differences between both groups in our trial.

Infusion therapy itself as well as surgical trauma can initiate a shedding of the endothelial glycocalyx [3,31]. This can lead to increased vascular permeability whereas different colloid solutions and coagulation factors are able to stabilize endothelial cell barrier functions. Pati et al. investigated effects of albumin solutions, fresh frozen plasma and prothrombin concentrates on trauma induced vascular integrity in human umbilical vein endothelial cells (HUVEC) and mice [32]. As a result, the authors showed an inhibition of endothelial damage by prothrombin concentrate and fresh frozen plasma, but not by albumin solution. However, in our investigation we found no differences regarding glycocalyx shedding between hydroxyethyl starch 6% (130/0.4) and albumin 5% while vascular integrity was not measured in our patients. Glycocalyx components are co-responsible for anticoagulant effects and fibrinolysis in experimental and clinical trials [12,13,33]. In our patients, serum hyaluronan decreased until end of surgery and afterwards increased in both groups until the 3rd postoperative day. One can speculate that this late increase reflects a pronounced degradation of the endothelial glycocalyx, induced by perioperative inflammation, whose maximum was masked by intraoperative hemodilution. This may also be valid for the course of perioperative syndecan-1 serum concentrations. However, especially hyaluronan is a rather unspecific variable, ubiquitously detectable in various soft tissues and, amongst other things, involved in cell proliferation and migration, inflammatory response and the development of autoimmune and malignant diseases [34–36]. At large, we did not observe explicit differences or – on the other hand - unidirectional tendencies in the perioperative course of the serum concentrations of all glycocalyx components. Therefore, our hypotheses concerning a relationship

Table 3
Rotational thrombelastometry readings and platelet function test results.

	Albumin (n = 28)	HES (n = 27)	p-Value
Thrombelastometry [% change]			
CT $_{INTEM}$	−3.3 (−5.8/+4.8)	+2.5 (−5.3/+12.1)	0.168
CFT $_{INTEM}$	+11.1 (+3.6/+16.9)	+21.6 (+8.6/+55)	0.021
A10 $_{INTEM}$	−2.4 (−4.2/ ± 0)	−5.7 (−12.7/−3.6)	0.004
A20 $_{INTEM}$	−1.7 (−3.8/ ± 0)	−5.1 (−10.3/−2.9)	0.002
MCF $_{INTEM}$	−1.7 (−3.8/+1.4)	−4.8 (−10.3/−3.1)	0.005
MCE $_{INTEM}$	−5.2 (−11.2/+1.3)	−12.9 (−25.6/−6.2)	0.006
CT $_{EXTEM}$	+10.2 (± 0/+14)	−4.2 (−7.3/+28.1)	0.570
CFT $_{EXTEM}$	+27.9 (+14.8/+47.1)	+34.7 (+21.4/+83.1)	0.126
A10 $_{EXTEM}$	−6.8 (−8.9/−1.9)	−11.3 (−17.6/−2.9)	0.056
A20 $_{EXTEM}$	−4.3 (−7.9/−1.4)	−10 (−13.8/−1.6)	0.052
MCF $_{EXTEM}$	−4.5 (−6.3/0)	−8.5 (−12.1/0)	0.046
MCE $_{EXTEM}$	−10.8 (−19.2/−0.5)	−23.9 (−33.5/−2.9)	0.035
CT $_{FIBTEM}$	+3.5 (−2/+7.1)	+2 (−7.4/+15.3)	0.980
A10 $_{FIBTEM}$	−23.2 (−32/−9.4)	−42.9 (−53.8/−30.4)	0.001
A20 $_{FIBTEM}$	−23.1 (−30.8/−12.5)	−41.7 (−50/−29.2)	0.002
MCF $_{FIBTEM}$	−23.1 (−32.1/−12)	−41.7 (−50/−30.8)	0.001
MCE $_{FIBTEM}$	−23.8 (−38.5/−12.9)	−50 (−56.3/−35.7)	0.001
Platelet function [% change]			
Multiplate TRAP	+6.5 (−7.6/+29.9)	−6.0 (−17.6/+17.1)	0.161
Multiplate ADP	−5.8 (−15/+9.3)	−6.0 (−31.1/+21.2)	0.860
Multiplate ASPI	−5.1 (−33.6/+19.2)	−13.5 (−44.8/+6.5)	0.471
PFA ADP	−11.1 (−21.1/ ± 0)	−3.1 (−18.4/+33.5)	0.337
PFA EPI	−9.2 (−32.1/+8.7)	−10.3 (−22.2/+25.9)	0.819

All values given as median (interquartile range). Percent change from beginning to end of surgery. HES = hydroxyethyl starch; CT = clotting time; CFT = clot formation time; A10 = amplitude after 10 min; A20 = amplitude after 20 min; MCF = maximum clot firmness; MCE = maximum clot elasticity; TRAP = thrombin receptor activating peptide; ADP = adenosine diphosphate; ASPI = arachidonic acid; EPI = epinephrine. Mann-Whitney-*U* test for metrical variables.

Table 4
Serum values of endothelial glycocalyx variables.

	Albumin (n = 28)	HES (n = 27)	p-Value
Syndecan-1 [ng/ml]			
Preoperative	27.9 (14.3/58.8)	17.2 (10.6/54.6)	0.257
End of surgery	21.4 (11.9/68.2)[#]	17.8 (10.3/45.7)	0.586
2 to 4 h after surgery	32.1 (11.6/64.9)	32.3 (18.3/71.9)[#]	0.480
1st POD	27.7 (12.1/61.3)	22.2 (10.8/74.6)	0.763
3rd POD	20.6 (8.5/83.1)	29.6 (17.3/70.8)	0.396
Hyaluronan [ng/ml]			
Preoperative	134.4 (109.2/160.2)	117.4 (98.2/153.8)	0.391
End of surgery	111.3 (91.5/139.7)[#]	102.6 (86.7/141.6)	0.625
2 to 4 h after surgery	133 (100.1/162.2)	128.8 (95.3/166)	0.880
1st POD	166.1 (142.2/191.5)[*]	160.8 (115.1/186.4)[*]	0.484
3rd POD	155.3 (131.9/243.8)[*]	178 (138.2/221.3)[*]	0.736
Heparan sulfate [ng/ml]			
Preoperative	1189.3 (998.2/1823.5)	1332.1 (956.1/1609.6)	0.960
End of surgery	1325.7 (817.8/1886.1)	1228.3 (933.4/1549.6)	0.866
2 to 4 h after surgery	1202.4 (886.9/1635.3)	1417.9 (945.9/1737.9)	0.590
1st POD	1232 (974.3/1583.8)	1309.7 (993.2/1555.5)	0.723
3rd POD	1245.8 (967.1/1946.9)	1203 (843.2/1797.6)	0.684

All values given as median (interquartile range). HES = hydroxyethyl starch. POD = postoperative day. Mann-Whitney-*U* test for metrical variables; two-way ANOVA with adjusting of alpha level for repeated measurements.
[#] Significant change from baseline ($p < 0.05$).
[*] Significant change from baseline ($p < 0.001$).

Table 5
Infusion requirements, blood loss, hemoglobin values and transfusion rates.

	Albumin (n = 28)	HES (n = 27)	p-Value
Intraoperative crystalloid requirements [ml]	2380 ± 810	2060 ± 650	0.257
Total crystalloid requirements[a] [ml]	10,630 ± 2900	11,060 ± 3170	0.409
Colloid requirements[b] [ml]			
Until transfer to ICU, PACU or ward	1260 ± 550	1590 ± 640	0.050
1st POD	50 ± 210	0 ± 20	0.549
2nd and 3rd POD	0 ± 0	0 ± 0	1.000
Calculated intraoperative blood loss [ml]	1245 ± 709	1557 ± 825	0.042
Transfusion rates, no. (%)			
Intraoperative			
Erythrocyte transfusion	7 (25.0)	7 (25.9)	0.999
Fresh frozen plasma transfusion	2 (7.1)	2 (7.4)	0.999
Platelet transfusion	0 (0)	0 (0)	
Until 1st POD			
Erythrocyte transfusion	2 (7.1)	4 (14.8)	0.422
Fresh frozen plasma transfusion	0 (0)	0 (0)	
Platelet transfusion	0 (0)	0 (0)	
Until 3rd POD			
Erythrocyte transfusion	3 (10.7)	3 (11.1)	0.999
Fresh frozen plasma transfusion	0 (0)	0 (0)	
Platelet transfusion	0 (0)	0 (0)	

Values given as mean ± SD. HES = hydroxyethyl starch; ICU = intensive care unit; PACU = postanesthesia care unit; POD = postoperative day. Mann-Whitney-*U* test for metrical variables; Fisher's exact *t*-test (two-sided) for dichotomous variables.
[a] Up to the 3rd POD.
[b] Without erythrocyte, fresh frozen plasma and platelet transfusion.

between glycocalyx shedding and an impairment of coagulation was not supported by the current data. On the contrary, it is more likely that the more pronounced impairment of clot firmness in the hydroxyethyl starch group was caused by an isolated effect of hydroxyethyl starch 6% (130/0.4). Primary hemostasis, evaluated by clotting time, was comparable in both groups, suggesting that reduced clot firmness in the hydroxyethyl starch group is possibly due to an induced fibrin polymerization disorder, which is caused not only by fibrinogen dilution but also by reduced FXIIIa-mediated fibrin cross linking [37,38]. However, data presented here are from a subgroup analysis and caution is appropriate when interpreting the results.

It is known from previous studies that calculation of blood loss is much more accurate than estimation [25,39]. Kadri et al. were able to show that physicians underestimate postpartum blood loss by about 30% in obstetric patients [39]. In addition, estimation of blood loss in our patients was difficult, due to the surgical method with opening of the bladder. The formula used to calculate blood loss requires normovolemia [25]. This is the case with most patients before elective surgery, as we have shown in earlier research [40]. Normovolemia was maintained by a standardized infusion and transfusion regimen based on invasive hemodynamic monitoring (see methods). The exact formula and its calculation were published previously and is nowadays routinely used in our institutional patient data management system [2].

Our investigation has some limitations. First, the current data are part of a subgroup analysis which included only 55 patients out of 100 in the overall population. Primary endpoint of the main trial was renal function but not the influence of colloidal solutions on coagulation. Blood loss in the overall population was estimated by anesthetists and surgeons but not calculated by a more accurate formula. This may be the reason for the obvious differences regarding intraoperative blood loss between the subgroup and the overall population in which we were not able to detect differences between both groups. In addition, there was a significant difference in age in this subgroup (albumin: 65 ± 9 ys vs HES: 70 ± 9 ys, p = 0.021). Due to the small number of cases, we did not do propensity score matching and instead performed a linear regression analysis. It was found that neither age nor other independent variables (preoperative medication, BMI, ASA classification) had any influence on intraoperative blood loss. Second, it is unclear whether the higher amount of administered colloid solution in the hydroxyethyl starch group is either the cause or the consequence of greater intraoperative blood losses. The conclusion whether the administration of hydroxyethyl starch increased blood loss or the higher blood loss resulted in a higher colloid amount is therefore not permissible. Third, we measured rotational thrombelastometry and platelet function only before and after surgery, but not on the 1st and 3rd postoperative day. This circumstance limits a comparison with and association to the course of glycocalyx components which were measured more frequently. Indeed, we found that the course of glycocalyx components was comparable in both groups until 3rd postoperative day. Therefore, degradation of endothelial glycocalyx seems not to be related to the significantly more pronounced impairment of variables of rotational thrombelastometry in the hydroxyethyl starch group.

5. Conclusion

In summary, numerous in vivo and in vitro studies have demonstrated the effects of hydroxyethyl starches on clot formation, clot firmness and platelet function. To the best of our knowledge, the data presented here are the first comparing balanced hydroxyethyl starch 6% (130/0.4) and albumin 5% in a perioperative setting to detect influences on coagulation using modern bedside point-of-care diagnostic. Albeit a shedding of the endothelial glycocalyx was correlated with coagulopathy in different previous clinical trials, we were not able to confirm these results. Nevertheless, the reduced clot firmness in the hydroxyethyl starch group indeed seemed to be associated with administration of hydroxyethyl starch solution. This finding is not only of scientific but of clinical relevance taking into account that the calculated blood loss of our study population was significantly higher in the hydroxyethyl starch group compared to the albumin group. However, the results presented derive from a subgroup analysis and must be considered with caution.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.thromres.2019.10.020.

Declarations

Ethical approvals were obtained from the Ethics Committee of the Ludwig-Maximilians-University of Munich (reference number: 311-11) and the responsible drug administration authority (Paul-Ehrlich-Institute of the German Federal Ministry of Health). For this subgroup analysis an amendment was written and approval from the Ethics Committee was given in March 2013. Written informed consent was obtained from all patients.

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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Authors' contributions

Tobias Kammerer, Simon Schäfer, Klaus Hoffmann-Kiefer, Peter Conzen and Markus Rehm: these authors have written the manuscript. Tobias Kammerer is the corresponding author. Tobias Kammerer, Nikolai Hulde, Eike Speck and Vera von Dossow: these authors have planned the trial and were responsible for patient recruitment and care. Max Hübner: this author was responsible for analysis of glycocalyx components and revised the manuscript. Bernhard Zwißler: this author was the principle investigator and gave substantial contributions to the conception of the work. Markus Rehm: this author was the sponsor delegated person of the trial. Alexander Crispin: this author has done statistical analyzes. Simon Schäfer: this author was mainly responsible for coagulation analyzes. All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no competing interests.

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