FACULTY OF HEALTH AND MEDICAL SCIENCES
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PhD thesis

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Hydroxyethyl Starch in Sepsis

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This thesis has been submitted to the Graduate School of The Faculty of Health and Medical Sciences, University of Copenhagen on the 22nd June 2013

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ORIGINAL PAPERS

The present PhD thesis is based on the following papers:

I. Perner A, **Haase N**, Guttormsen AB, Tenhunen J, Klemenzson G, Åneman A, Madsen KR, Møller MH, Elkjær JM, Poulsen LM, Bendtsen A, Winding R, Steensen M, Berezowicz P, Søe-Jensen P, Bestle M, Strand K, Wiis J, White JO, Thornberg KJ, Quist L, Nielsen J, Andersen LH, Holst LB, Thormar K, Kjældgaard A-L, Fabritius ML, Mondrup F, Pott FC, Møller TP, Winkel P, Wetterslev J. Hydroxyethyl starch 130/0.42 versus Ringer's acetate in severe sepsis. N Engl J Med 2012; 367: 124–34

II. **Haase N**, Wetterslev J, Winkel P, Perner A on behalf of the 6S trial group and the Scandinavian Critical Care Trial Group. Bleeding and Risk of Death with Hydroxyethyl Starch in Severe Sepsis. Manuscript submitted.

III. **Haase N**, Perner A, Hennings LI, Siegemund M, Lauridsen B, Wetterslev M, Wetterslev J. Hydroxyethyl starch 130/0.38-0.45 versus crystalloid or albumin in patients with sepsis: systematic review with meta-analysis and trial sequential analysis. BMJ 2013; 346: f839.

PREFACE

This thesis is based on two studies performed during my employment at the Department of Intensive Care, Copenhagen University Hospital, Rigshospitalet, in the period 2009 to 2013.

I would like to thank all the great and dedicated people, who have contributed to this thesis and/or supported me during the process. First and foremost, I would like to express my gratitude to my supervisors Anders Perner, Jørn Wetterslev and Pär Johansson for constant support, constructive criticism, rewarding discussions and their strive for the highest scientific standards. I cannot thank you enough for entrusting me with the 6S trial and for giving me the experiences that came along with it.

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The studies were supported by the Danish Research Councils, the Rigshospitalet Research Council and the Scandinavian Society of Anesthesiology and Intensive Care Medicine to whom I am grateful.

Finally, I wish to thank my wonderful wife and son for love, support and our many scientific discussions across the dining table.

Nicolai Rosenkrantz Segelcke Haase

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SUMMARY

Background

Hydroxyethyl starch (HES) is a colloid that has been widely used for fluid resuscitation for decades. The newest generation of HES, tetrastarch, was believed to provide an efficient volume expansion without causing the side effects observed with former HES solutions. However, this belief was based on physiological models and small studies rather than on firm clinical evidence.

Our aim was to assess the safety and efficacy of tetrastarch in a randomised clinical trial and in a systematic review.

Methods

We first conducted a blinded, clinical trial, in which we randomly assigned patients with severe sepsis in the intensive care unit to fluid resuscitation with either 6% HES 130/0.42 (Tetraspan) or Ringer's acetate. The primary outcome measure was death or dialysis-dependency at 90 days after randomisation. Secondary outcomes described kidney function and serious adverse reactions.

Secondly, we systematically identified all randomised clinical trials comparing tetrastarch with either crystalloid or albumin in patients with sepsis and pooled their results in meta-analyses and trial sequential analyses.

Results

Of the 804 patients who underwent randomisation, 798 were included in the modified-intention-to-treat population. At 90 days after randomisation, 201 of 398 patients (51%) assigned to HES 130/0.42 had died, as compared with 172 of 400 patients (43%) assigned to Ringer's acetate (relative risk 1.17, P=0.03); 1 patient in each group was dialysis-dependent at 90 days. In the 90 day observation period, 87 patients (22%) assigned to HES received renal replacement therapy vs. 65 patients (16%) assigned to Ringer's acetate (relative risk 1.35, P=0.04), and 38 patients (10%) vs. 25 patients (6%) had severe bleeding (relative risk 1.52, P=0.09). Post-hoc sensitivity analysis showed a strongly significant increased risk of any bleeding with HES vs. Ringer's acetate (relative risk 1.56, P=0.003).

In the systematic review, we identified nine trials that randomised 3,456 patients with sepsis. In meta-analyses, tetrastarch vs. crystalloid or albumin lead to increased use of renal replacement therapy (relative risk 1.36, P=0.009) and red blood cells (relative risk 1.29, P=0.0002)

and to more serious adverse events (relative risk 1.30, P=0.03). Trials with low risk of bias suggested 11% increased risk of death. After adjusting the results with trial sequential analysis signals for harm persisted.

Conclusion

Our randomised clinical trial is one of several high-quality trials in critically ill patients with and without sepsis that now provide evidence that the use of tetrastarch impairs kidney function and hemostasis and may even increase mortality. Whether the results can be extrapolated to other types of patients is unclear, but so far no group of patients with an overall benefit of HES beyond surrogate markers has been identified. In line with this, the European Medicines Agency's Pharmacovigilance Risk Assessment Committee now recommends that the marketing authorisations of all HES solutions are suspended in the European Union.

DANSK RESUMÉ

Baggrund

Hydroxyethyl stivelse (HES) er et kolloid, som i årtier har været anvendt til væskebehandling af kritisk syge patienter. Den nyeste generation af HES-produkter (tetrastarch) blev anset for at være effektiv og uden de bivirkninger man havde set med tidligere generationer af HES, men den antagelse var baseret på fysiologiske betragtninger og resultater fra små studier frem for på solid klinisk evidens.

Vores mål var at undersøge effekten og sikkerheden ved den nye generation af HES-produkter i et randomiseret klinisk forsøg og i en systematisk litteraturgennemgang.

Metoder

Vi gennemførte først et blindet, klinisk forsøg, hvor vi randomiserede patienter med svær sepsis indlagt på intensivafdeling til væskebehandling med enten 6% HES 130/0,42 (Tetraspan) eller Ringer acetat. Det primære effektmål var død eller afhængighed af dialyse 90 dage efter randomisering i forsøget. Sekundære effektmål belyste nyrefunktion og alvorlige bivirkninger.

Herefter lavede vi en systematisk litteraturgennemgang, hvor vi fandt alle randomiserede kliniske forsøg, der sammenlignede den nyeste generation af HES-produkter med krystalloid eller albumin i patienter med sepsis. Vi lavede en samlet analyse af forsøgene med konventionel metaanalyse og belyste den statistiske usikkerhed med 'trial sequential analysis'.

Resultater

798 af 804 randomiserede patienter kunne indgå i analysen. 90 dage efter randomisering var 201 af 398 patienter (51%) i HES-gruppen døde mod 172 af 400 patienter (43%) i Ringer acetat-gruppen (relativ risiko 1.17, P=0.03); én patient i hver gruppe var dialyse-krævende på dag 90. I opfølgningsperioden på 90 dage blev 87 patienter (22%) i HES-gruppen behandlet med dialyse mod 65 patienter (16%) i Ringer acetat-gruppen (relativ risiko 1.35, P=0.04), og henholdsvis 38 (10%) og 25 (6%) patienter havde en svær blødning (relativ risiko 1.52, P=0.09). En supplerende analyse af alle blødninger uafhængigt af sværhedsgraden viste, at HES-patienterne havde en kraftigt forøget risiko for blødning (relativ risiko 1.56, P=0.003).

I den systematiske litteraturgennemgang fandt vi 9 forsøg, der til sammen havde inkluderet 3.456 patienter med sepsis. I metaanalyser af forsøgene medførte den nyeste generation af HES-

produkter øget brug af dialyse (relativ risiko 1.36, P=0.009), flere blodtransfusioner (relativ risiko 1.29, P=0.0002) og flere alvorlige bivirkninger (relativ risiko 1.30, P=0.03) sammenlignet med krystalloid eller albumin. De forsøg, der var af høj kvalitet, antydede en øget risiko for død på 11%. Da vi havde taget højde for statistisk usikkerhed med 'trial sequential analysis' fremstod HES fortsat som potentielt skadeligt i patienter med sepsis.

Konklusion

Vores randomiserede kliniske forsøg er et af flere forsøg af høj kvalitet i kritisk syge patienter både med og uden sepsis, der nu dokumenterer, at den nyeste generation af HES-produkter giver nyreskade, blødningsforstyrrelser og sandsynligvis øget dødelighed. Hvorvidt resultaterne kan overføres til andre typer patienter er uklart, men det er endnu ikke lykkes at finde en patientgruppe med en samlet gavn af HES. Som følge af dette har Komiteen for Lægemiddelovervågning ved den Europæiske Lægemiddelstyrelse netop anbefalet, at alle HES-opløsninger trækkes af markedet i den Europæiske Union.

INTRODUCTION

The new generation of hydroxyethyl starch (HES), tetrastarch, was launched in 1999 as a promising colloid for restoration of circulation without the side effects observed with former HES solutions. It turned into one of the most used fluids world-wide with tens of millions patients treated each year and yearly sales exceeding hundreds of millions of dollars [1]. However, the belief in tetrastarch was based on physiological models and small studies rather than on firm clinical evidence.

This thesis is based on a randomised clinical trial and a systematic review in which we assessed the safety and efficacy of tetrastarch vs. other fluids in patients with sepsis. The thesis contains study descriptions and a discussion of their methods. Finally, the evidence for the use of HES is discussed.

BACKGROUND

Sepsis

Sepsis is a medical condition characterised by systemic inflammation as a response to infection. The disease may deteriorate to severe sepsis defined as sepsis with acute organ failure and to septic shock with hypotension that is not reversed by initial fluid therapy. Mortality rates depend on severity, but may be as high as 50%. Because several million people world-wide are affected each year, sepsis is a leading cause of death and a burden to society [2–5].

The systemic inflammation in sepsis affects the cardiovascular system causing loss of vascular tone, capillary leakage and depressed cardiac function all leading to circulatory failure with organ hypoperfusion and eventually death [6]. Resuscitation with infusion of fluid to increase the intravascular volume is life-saving in these patients and constitutes a cornerstone in the treatment of patients with sepsis in the intensive care unit (ICU).

Hydroxyethyl Starch

Fluids for medical use are divided into two categories: The crystalloids consisting of mineral salts and water, and the colloids, where large insoluble molecules have been added to the fluid. HES solutions are colloids and consist of large hydroxyethylated starch molecules dispersed into a carrier solution of water and mineral salts. Derived from maize or potatoes they are cheap, synthetic alternatives to the natural colloid, albumin. The solutions are polydisperse and

characterised by their mean molecular weight, degree of hydroxyethylation (substitution ratio) and C2:C6 pattern for hydroxyethylation (figure 1). Most commonly HES is referred to as HES 200/0.5, HES 130/0.4 or similar, where the first number is the molecular weight and the second the substitution ratio.

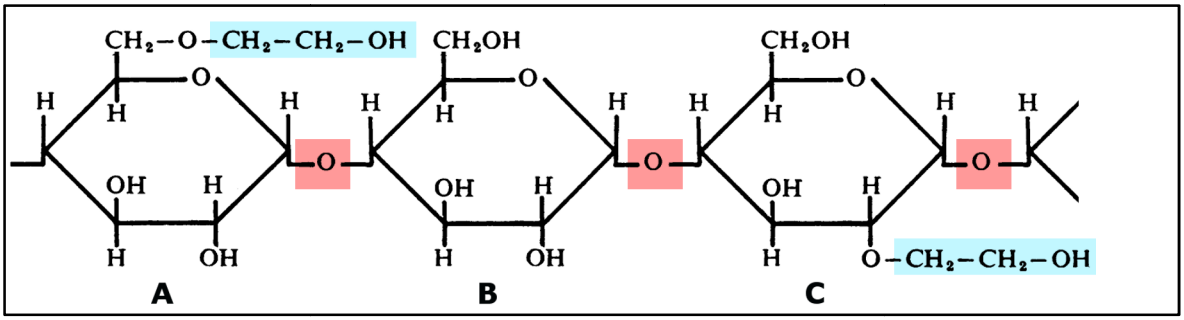

Figure 1 Simplified structure of HES. The figure shows a chain of glucose units (labelled A, B and C) with hydroxyethyl groups attached (blue). Two of three glucose units are hydroxyethylated. Hydroxyethyl is bound to C6 at glucose unit A and to C2 at glucose unit C. Amylase splits the chain of glucose at the binding sites marked with red. C2- vs. C6-hydroxyethylation reduces the enzymatic breakdown of HES. Modified from [7].

HES is metabolised by endogenous amylases, which break down the starch molecules into smaller molecules that are filtered in the glomerulus and excreted in the urine. Faecal excretion is negligible. The substitution ratio is the main determinant of degradation, where a high ratio slows down metabolisation leaving larger molecules in the blood stream for longer. Similarly, C2- vs. C6-hydroxyethylation reduces the enzymatic breakdown of HES [7–10].

Since the first HES solution was introduced around 1970, other easier degradable solutions with lower molecular weights and lower substitution ratios have been introduced. In the last decade, HES 130/0.4 and HES 130/0.42 have replaced most other HES solutions. Derived from their substitution ratio their common name is ‘tetrastarch’.

Hydroxyethyl Starch in Sepsis

According to simple physiological models of fluid compartments and membranes, colloid solutions such as HES should be preferred over crystalloids, because the large colloid molecules remain in the intravascular space, where they retain water that would otherwise diffuse into the tissue and cause oedema (figure 2). In alignment with this, medical textbooks often state that 3 litre of crystalloid is needed to obtain the same increase in intravascular volume as that of 1 litre of

colloid [11]. Thus, theoretically, the use of HES may efficiently improve circulation without causing oedema and fluid overload, which are associated with organ failure and death [12, 13].

In 2009, when we designed the present PhD study, the clinical use of HES in sepsis was debated, because two randomised clinical trials showed increased risk of acute kidney injury with HES 200/0.5-0.6 in patients with sepsis [3, 14]. In other types of patients, HES was associated with hemostatic impairment [15], persistent pruritus [16] as well as deposition of HES particles in macrophages [17] and in multiple organs including the liver, kidney, skin, intestine, striated muscle, spleen and placenta [17–21].

The implication of these findings for the use of tetrastarch remained unclear. Some claimed that tetrastarch provided efficient volume expansion without the side effects observed with former HES solutions, because its elimination was faster [8]. Others claimed that tetrastarch was tested only in small studies inadequately designed to establish its efficacy and safety [22–24].

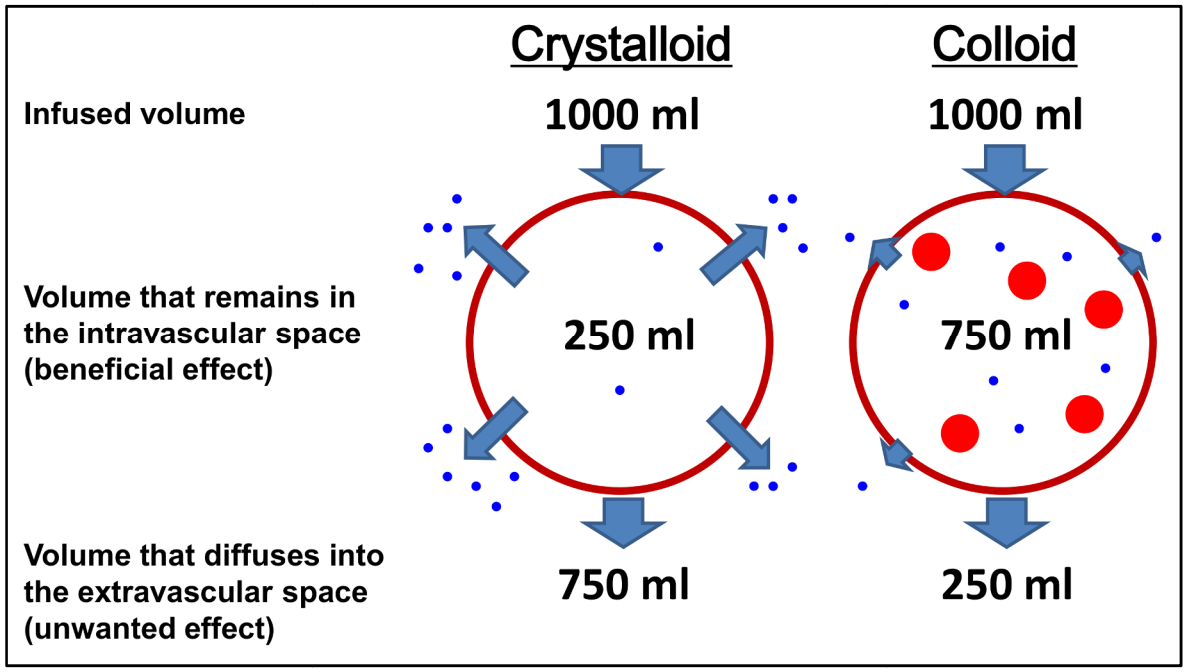

Figure 2 A simple physiological model of fluid compartments and membranes constituting the rationale for the use of colloids instead of crystalloids. The crystalloids consist of small molecules (blue), which diffuse across the endothelial barrier to the extracellular space and draw water with them. The colloids contain larger molecules (red), which, according to the model, remain in the intravascular space and retain water. Thus, theoretically, colloids are three times more potent than crystalloids and cause less oedema.

Despite these controversies, tetrastarch was the most commonly used colloid for resuscitation of critically ill patients in ICUs both in Scandinavia and world-wide [25, 26] and the use was increasing (sales figures of Voluven, Fresenius Kabi, provided by Christiane Hartog). Thus, studies assessing the clinical effects of tetrastarch on patient-important endpoints were urgently needed.

AIM OF STUDIES

Our primary aim was to investigate the effects of tetrastarch vs. crystalloid on mortality, kidney function and serious adverse reactions in patients with severe sepsis in a randomised clinical trial. Secondarily, we aimed at comparing our results with those of similar trials in a systematic review.

STUDY OUTLINE

The present PhD thesis is based on two studies and three papers:

Study I is the Scandinavian Starch for Severe Sepsis / Septic Shock (6S) Trial – a blinded, multicenter, randomised clinical trial assessing the effects of HES 130/0.42 vs. Ringer's acetate in patients with severe sepsis. Paper I is the main publication of the trial, which presents the results on mortality, kidney function, serious adverse reactions and other pre-defined outcomes. Paper II contains post-hoc analyses of the relationships among type of trial fluid, haemostatic variables, bleeding and mortality.

Study II is a systematic review of randomised clinical trials comparing tetrastarch vs. crystalloid or albumin in patients with sepsis. This study is presented in paper III.

STUDY I: THE SCANDINAVIAN STARCH FOR SEVERE SEPSIS / SEPTIC SHOCK TRIAL

Methods

Overview and design

The Scandinavian Starch for Severe Sepsis / Septic Shock (6S) trial was a multicenter, blinded, parallel-group clinical trial, which randomised patients from 23rd December 2009 to 18th November 2011 in 26 ICUs in Denmark, Norway, Finland and Iceland. Randomisation was centralised and blinded with stratification according to the presence of shock, presence of

haematological malignancy and admission to a university vs. non-university hospital. Written informed consent was obtained from the patients and/or their legal substitute prior to randomisation.

The trial was approved by the Ethics Committees, Medicines Agencies and Data Protection Agencies in the participating countries and registered at the clinicaltrials.gov website (NCT00962156). The trial protocol and statistical analysis plan were published before end of trial [27, 28].

Patients

Adult patients in the ICU with severe sepsis were eligible for randomisation, if the clinician judged that the patient needed fluid resuscitation. Exclusion criteria included renal replacement therapy, intracranial haemorrhage during current hospitalisation and treatment with >1,000 ml of synthetic colloid in the 24 hours prior to assessment for eligibility.

Intervention

Patients were assigned 1:1 to fluid resuscitation with either 6% HES 130/0.42 in Ringer's acetate (6% Tetraspan, B Braun Medical) or Ringer's acetate (Sterofundin, B Braun Medical). Trial fluid in sealed, opaque plastic bags was used for fluid resuscitation in the ICU for a maximum of 90 days and was given at the discretion of the clinician to a maximum daily dose of 33 ml/kg ideal body weight. Open-labelled Ringer's acetate was used thereafter. All other interventions were at the discretion of the clinicians. If patients developed severe allergic reactions, severe bleedings (intracranial bleeding or bleeding with concomitant transfusion with 3 units of red blood cells) or need of renal replacement therapy, treatment with trial fluid was permanently stopped and saline or Ringer's lactate was given during the remaining trial period.

Treatment allocation was concealed for patients, clinicians, nursing and research staff and the statistician.

Outcomes

The composite primary outcome measure was death or dependence on dialysis 90 days after randomisation. Secondary outcomes described among other things kidney function and serious adverse reactions (table 1).

Statistical analysis

The modified intention-to-treat population was analysed for difference between groups with chi-square and Wilcoxon rank-sum test where appropriate. Cox regression and uni- and multivariate logistic regression analyses were used for post-hoc outcomes. We used area under the curve and mixed models in the analyses of changes in haemostatic variables over time. P values lower than 0.05 were considered statistically significant.

Results

Of 1,211 patients evaluated for inclusion 804 underwent randomisation and 798 patients were included in the modified intention-to-treat analysis (figure 3). Baseline characteristics were similar in the intervention groups.

Fluid therapy, use of blood products and circulatory effects

The median cumulative dose of blinded trial fluid was 3,000 ml (interquartile range 1,507 to 5,100) corresponding to 44 ml per kilo ideal body weight in the HES group and 3,000 ml (interquartile range 2,000-5,750) corresponding to 47 ml per kilo in the Ringer's acetate group. More patients in the HES vs. Ringer's acetate group were transfused with red blood cells (relative risk 1.28, 95%-CI 1.12 to 1.47, P<0.001). Circulatory variables in the first 24 hours after randomisation did not differ significantly between the groups.

Predefined outcomes

Primary outcome and mortality

202 (51%) patients in the HES group and 173 (43%) patients in the Ringer's acetate group fulfilled the primary outcome, death or dialysis dependency 90 days after randomisation (relative risk 1.17, 95%-CI 1.01-1.36, P=0.03). As only one patient in each group was dependent on dialysis, the difference was due to increased risk of death at day 90 with HES. The findings were supported by multivariate analyses with adjustment for known baseline risk factors.

The separation of the survival curves occurred approximately from day 20 to day 60 where after the survival curves ran parallel (figure 4). The increased risk of death with HES persisted after one year, but the group difference was no longer statistically significant (relative risk 1.09, 95%-CI 0.96-1.24, P=0.20) (unpublished data).

Predefined subgroup analyses did not reveal statistically significant interaction between occurrence of the primary outcome and having acute kidney injury or septic shock at the time of randomisation.

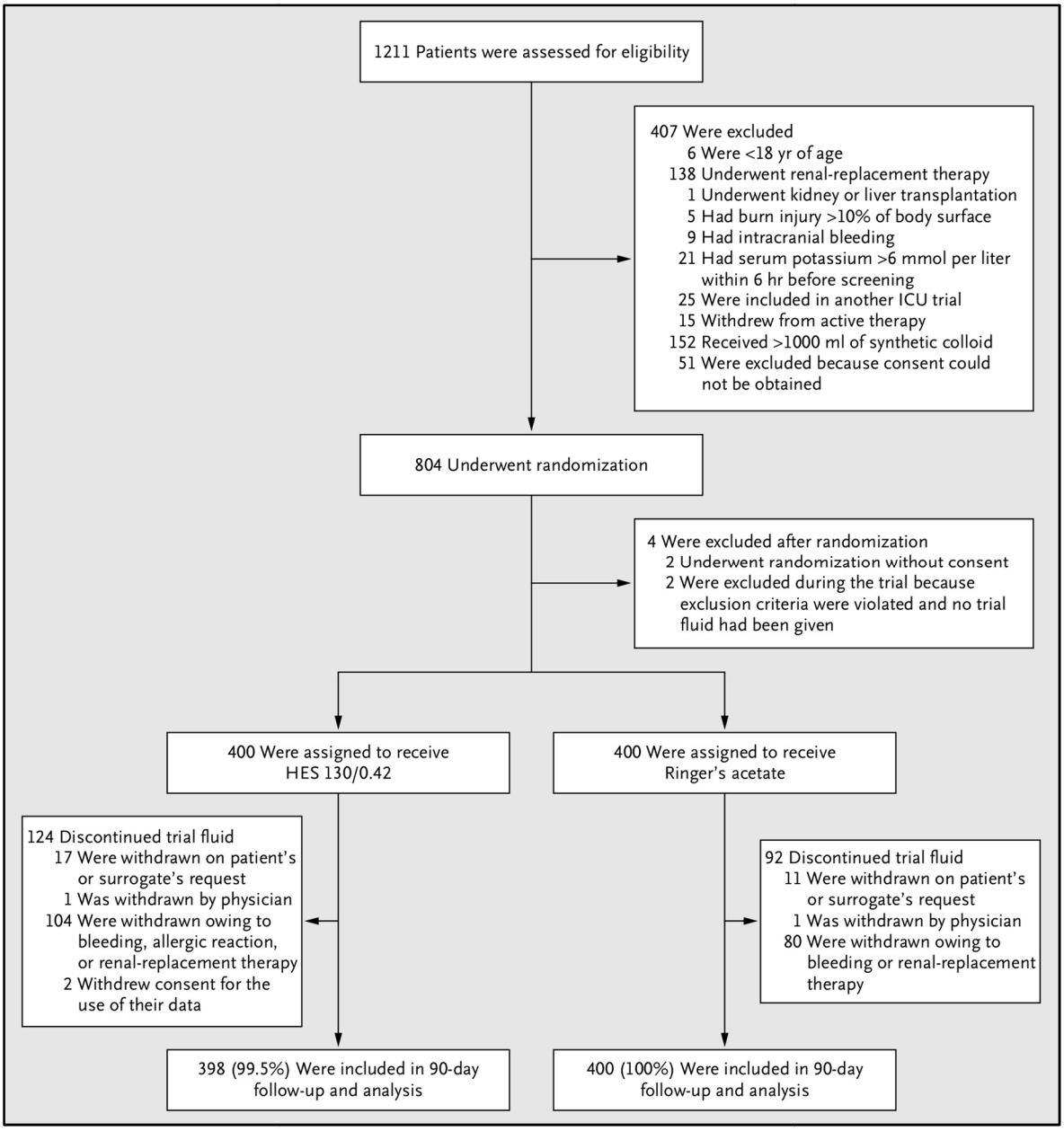

Figure 3 CONSORT diagram

Renal function

Patients assigned to HES vs. Ringer's acetate had increased use of renal replacement therapy and fewer days off dialysis during the 90-day follow-up (table 1). The incidences of acute kidney injury

(defined as renal replacement therapy or more than three points in the renal component of the Sepsis-related Organ Failure Assessment (SOFA) score [29]) and doubling of creatinine did not differ significantly between the groups, but the estimates favoured Ringer's acetate.

Table 1 Primary and Secondary Outcomes

Outcome	HES 130/0.42 (N=398)	Ringer's Acetate (N=400)	Relative Risk (95% CI)	P Value
Primary outcome				
Dead or dependent on dialysis at day 90 — no. (%)	202 (51)	173 (43)	1.17 (1.01–1.36)	0.03
Dead at day 90 — no. (%)	201 (51)	172 (43)	1.17 (1.01–1.36)	0.03
Dependent on dialysis at day 90 — no. (%)	1 (0.25)	1 (0.25)	—	1.00
Secondary outcome measures				
Dead at day 28 — no. (%)	154 (39)	144 (36)	1.08 (0.90–1.28)	0.43
Severe bleeding — no. (%)	38 (10)	25 (6)	1.52 (0.94–2.48)	0.09
Severe allergic reaction — no. (%)	1 (0.25)	0	—	0.32
SOFA score at day 5 — median (interquartile range)	6 (2–11)	6 (0–10)	—	0.64
Use of renal-replacement therapy — no. (%)	87 (22)	65 (16)	1.35 (1.01–1.80)	0.04
Use of renal-replacement therapy or renal SOFA score ≥3 — no. (%)	129 (32)	108 (27)	1.20 (0.97–1.48)	0.10
Doubling of plasma creatinine level — no. (%)	148 (41)	127 (35)	1.18 (0.98–1.43)	0.08
Acidosis — no. (%)	307 (77)	312 (78)	0.99 (0.92–1.06)	0.72
Alive without renal-replacement therapy — mean % of days	91	93	—	0.048
Use of mechanical ventilation — no. (%)	325 (82)	321 (80)	1.02 (0.95–1.09)	0.61
Alive without mechanical ventilation — mean % of days	62	65	—	0.28
Alive and out of hospital — mean % of days	29	34	—	0.048

Serious adverse reactions

Severe bleeding occurred in 38 (10%) patients in the HES group and 25 (6%) patients in the Ringer's acetate group. One patient in the HES group had a severe allergic reaction. No suspected unexpected serious adverse reaction (SUSAR) was observed.

Other pre-defined outcomes

Patients assigned to HES had fewer days alive and out of hospital during the 90-day follow-up compared to those treated with Ringer's acetate. There were no group differences in acidosis in the ICU, days off the ventilator and SOFA score 5 days after randomisation (table 1).

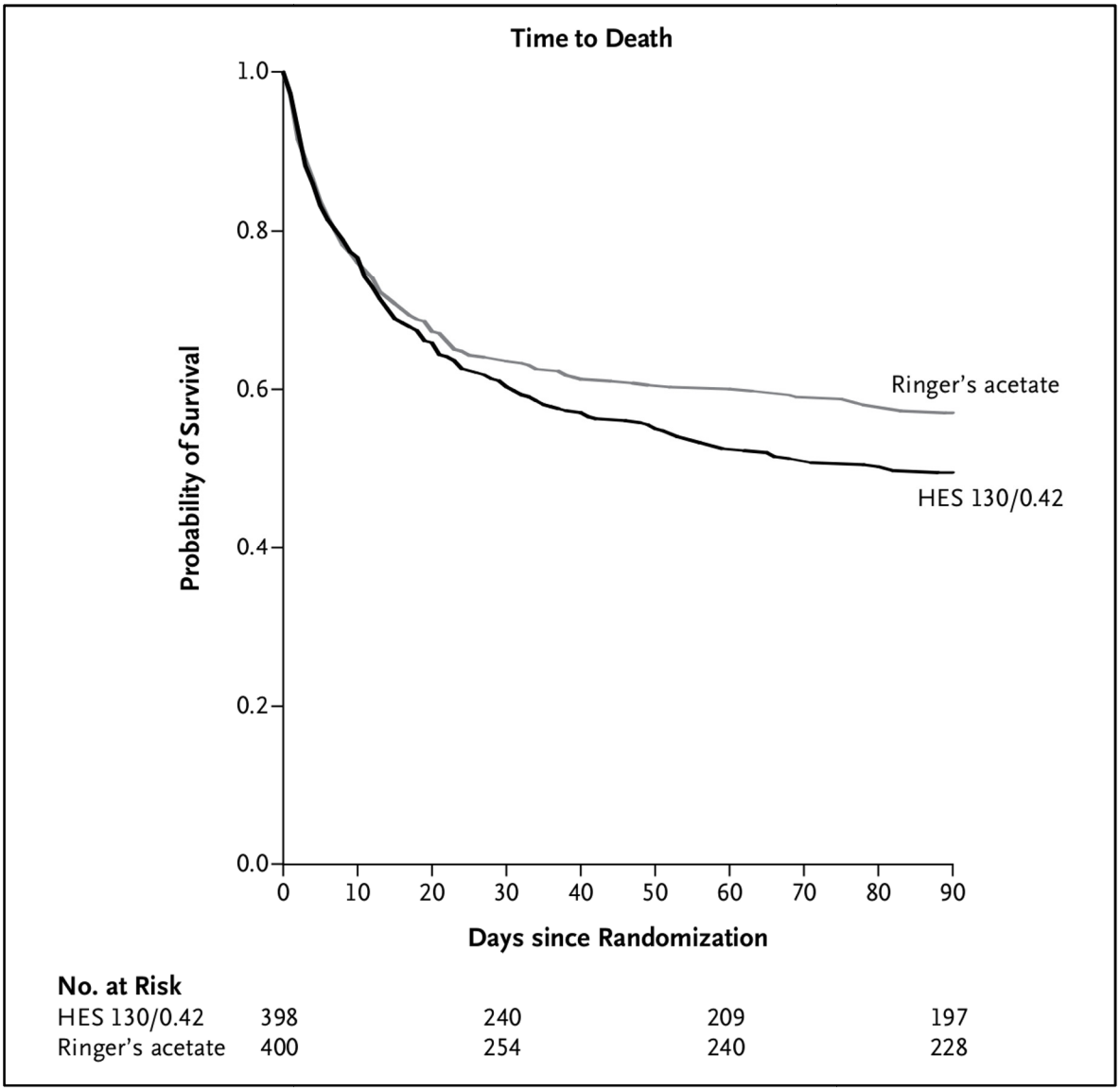

Figure 4 Survival curves censored at day 90 for the 798 patients in the modified-intention-to-treat population. Kaplan-Meier analysis showed that the survival time did not differ significantly between the two intervention groups (P=0.07)

Post-hoc analyses of the relationships among type of trial fluid, haemostatic variables, bleeding and mortality

Time course of INR, haemoglobin level and platelet count

Patients assigned to HES had statistically significant lower haemoglobin and higher INR values than those assigned to Ringer's acetate (figure 5). The differences occurred during the first days

after randomisation and seemed to diminish towards day 5. The platelet count was not affected with statistical significance by the type of trial fluid.

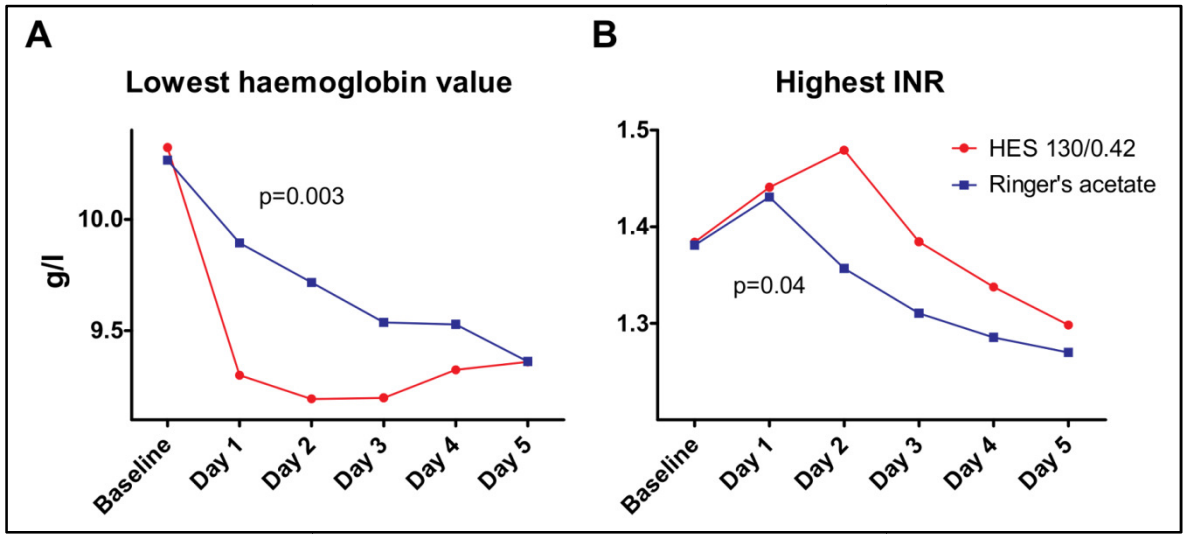

Figure 5 Time course of lowest Hemoglobin value (panel A) and of highest International Normalised Ratio (INR) (panel B) from baseline till five days after Randomisation. The curves show median values for each treatment group. P values are for differences in area under the curve.

Location, rates and timing of bleeding

Significantly more patients in the HES group had a bleeding episode compared to those in the Ringer's group (93 vs. 60 patients, relative risk 1.56, 95%-CI 1.16-2.08, P=0.003). The patients bleed mainly from wounds, from the upper gastrointestinal tract or during surgery. The majority of patients had their first bleeding episode within the first three days after randomisation (day 1: 33%; day 2: 15%; day 3: 7%), where most trial fluid was given. Once a bleeding episode occurred, its corresponding estimated blood loss was comparable between the groups (median 600 vs. 800 ml, P=0.31). The hazard ratio for any bleeding with HES was strongly statistically significant (HR 1.70, 95%-CI 1.23-2.36, P=0.001) (figure 6). The hazard ratios for severe bleeding with HES were comparable to those of any bleeding, but were not statistically significant (HR 1.55, 95%-CI 0.93-2.56, P=0.09).

Risk factors for bleeding

Fluid resuscitation with HES was an independent risk factor for bleeding. Other risk factors appeared to be admission to a university hospital, surgery prior to ICU admission and low platelet count.

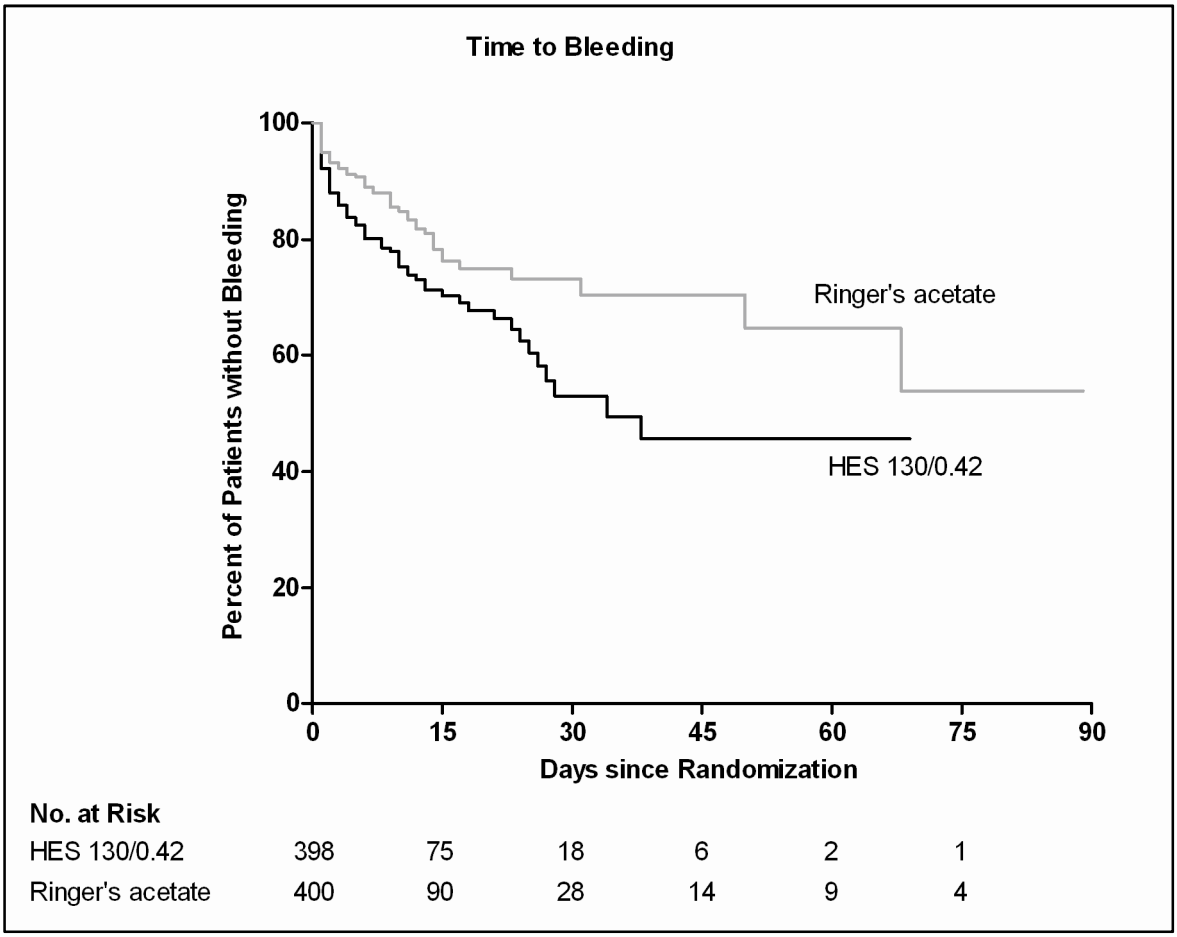

Figure 6 Kaplan-Meier curves of time to bleeding censored at death, discharge from the intensive care unit or at 90 days whichever came first for the two intervention groups. Kaplan-Meier analysis showed that the time to bleeding differed significantly between the groups (P=0.001).

Bleeding and death

The risk of death was significantly increased among patients with any bleeding and severe bleeding compared to those who did not bleed in the ICU in both unadjusted and adjusted analyses (figure 7).

Conclusion

The use of HES 130/0.42 vs. Ringer's acetate increased mortality at 90 days and patients assigned to HES were more likely to have renal replacement therapy and bleedings both of which associated with mortality.

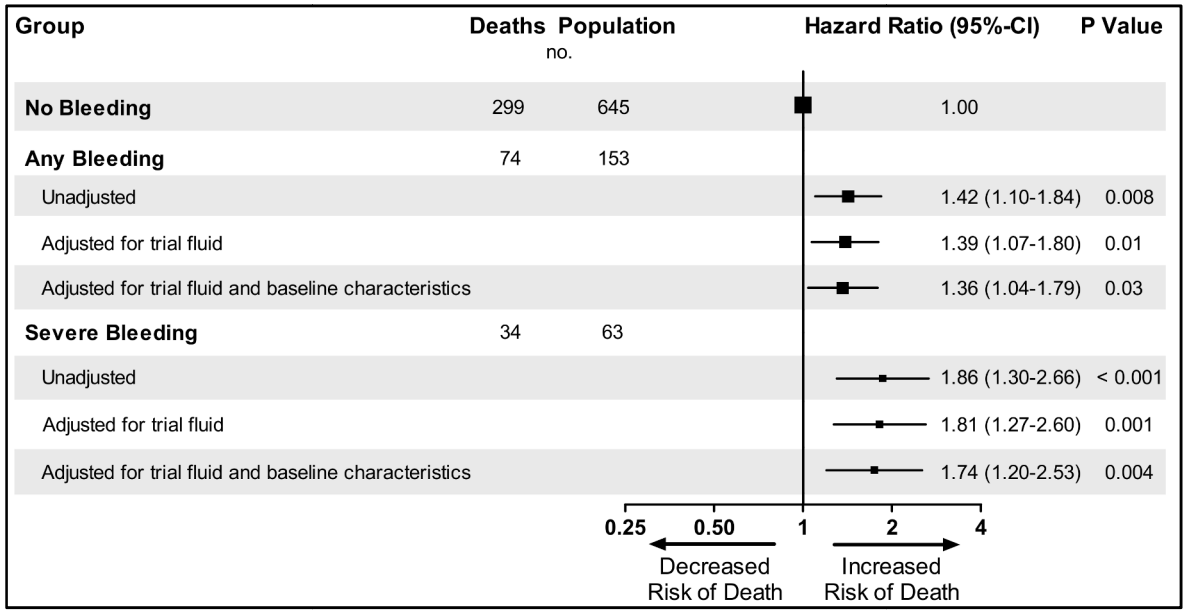

Figure 7 Hazard Ratio for Death According to Occurrence of any Bleeding or Severe Bleeding.

STUDY II: HYDROXYETHYL STARCH 130/0.38-0.45 VERSUS CRYSTALLOID OR ALBUMIN IN PATIENTS WITH SEPSIS: SYSTEMATIC REVIEW WITH META-ANALYSIS AND TRIAL SEQUENTIAL ANALYSIS

Methods

Overview

The systematic review was done in accordance with the recommendations from the Cochrane Collaboration [30] and a pre-published protocol [31].

Eligibility criteria

We searched for randomised clinical trials comparing tetrastarch (molecular weight 130 kDa and substitution ratio within the range of 0.38 to 0.45) with either crystalloid or albumin in patients with sepsis. Trials were included regardless of publication language or status. We included subgroups of septic patients from trials, if the randomisation was stratified for presence of sepsis or if the subgroup consisted of more than 500 septic patients. Cross-over studies were excluded.

Search strategy

Trials from 1995 and onwards were sought in the Cochrane Central Register of Controlled Trials, Medline, Embase, Biosis Previews, Science Citation Index Expanded and Cumulative Index to

Nursing, Allied Health Literature and clinical trial registries. Hand search included contact to manufacturers of HES and review of other systematic reviews.

Study selection, data extraction and risk of bias assessment

Study selection and data extraction was done independently by two persons. Co-primary outcomes were all-cause mortality and renal replacement therapy at end of follow-up. Secondary outcomes assessed renal function, coagulation, transfusion and serious adverse events (table 2). Risk of bias was evaluated according to pre-defined domains.

Statistical analyses

We used conventional meta-analytic statistics in the calculation of pooled estimates of the intervention effects. Trial Sequential Analysis correcting for sparse data and repetitive testing was used in the evaluation of the robustness of the results.

Results

Characteristics of included trials

Nine randomised clinical trials enrolling 3,456 patients were included, which included a subgroup of 1,937 patients from one trial. The included trials were heterogeneous in terms of diagnostic group, tetrastarch solution, comparator fluid and dosage. Four trials had low risk of bias in all domains, while the remaining trials had high risk of bias due to lack of blinding, vested financial interests or academic bias.

The observation periods varied from 24 hours to one year. In general, trials with low risk of bias had longer observation periods than those with high risk of bias.

Outcomes

All cause mortality

In the analysis of all trials contributing with mortality data, there was no overall mortality difference between patients treated with tetrastarch vs. crystalloid or albumin (table 2). However, trials with low risk of bias indicated 11% increased mortality with HES. Trial sequential analysis of trials with low risk of bias showed that there was no firm evidence for harm, but that HES would unlikely show large mortality benefit, if further adequately designed trials would be conducted in the future and added to the meta-analysis.

Table 2 Results from the Conventional Meta-analyses and Trial Sequential Analyses (TSA)

Outcomes	**No. of trials**	**HES group** events / total	**Control group** events / total	**Relative risk** (95%-CI)	**TSA adjusted 95%-CI**
Mortality					
All trials	8	552/1741	505/1673	1.04 (0.89-1.22)	0.70-1.54
Low risk of bias	4	499/1517	450/1499	1.11 (1.00-1.23)	0.95-1.29
Follow-up > 28 d	4	533/1591	478/1565	1.11 (1.01-1.22)	0.95-1.29
Renal Effects					
Renal replacement therapy	5	136/650	101/661	1.36 (1.08-1.72)	1.03-1.80
Doubling of creatinine	4	172/492	148/502	1.18 (0.99-1.40)	0.90-1.54
Haemostasis					
Transfusion with red blood cells	3	251/486	195/487	1.29 (1.13-1.48)	1.10-1.51
Bleeding	2	102/498	70/495	1.34 (0.81-2.21)	-
Serious Adverse Events					
	4	100/533	76/536	1.30 (1.02-1.67)	0.93-1.83

Blood loss and transfused volume of blood did not differ between the groups (data not shown).

Renal function

Tetrastarch significantly increased the risk of having renal replacement therapy by 36% (p=0.009). In alignment with this, we found a trend towards more patients having acute kidney injury defined as a doubling of creatinine (P=0.07). According to trial sequential analysis, the meta-analysis of renal replacement therapy provided firm evidence for increased use in the HES group (figure 8).

Transfusion with red blood cells, bleeding and blood loss

Tetrastarch significantly increased the risk of being transfused with red blood cells in the ICU in both conventional meta-analysis (P=0.0002) and trial sequential analysis. There were no group difference regarding volumes of transfused blood, blood loss and bleeding.

Serious adverse events

Serious adverse events were defined differently between trials, but patients assigned to tetrastarch had overall more serious adverse events than those assigned to control fluid (P=0.03). More data, however, would be needed to confirm this finding.

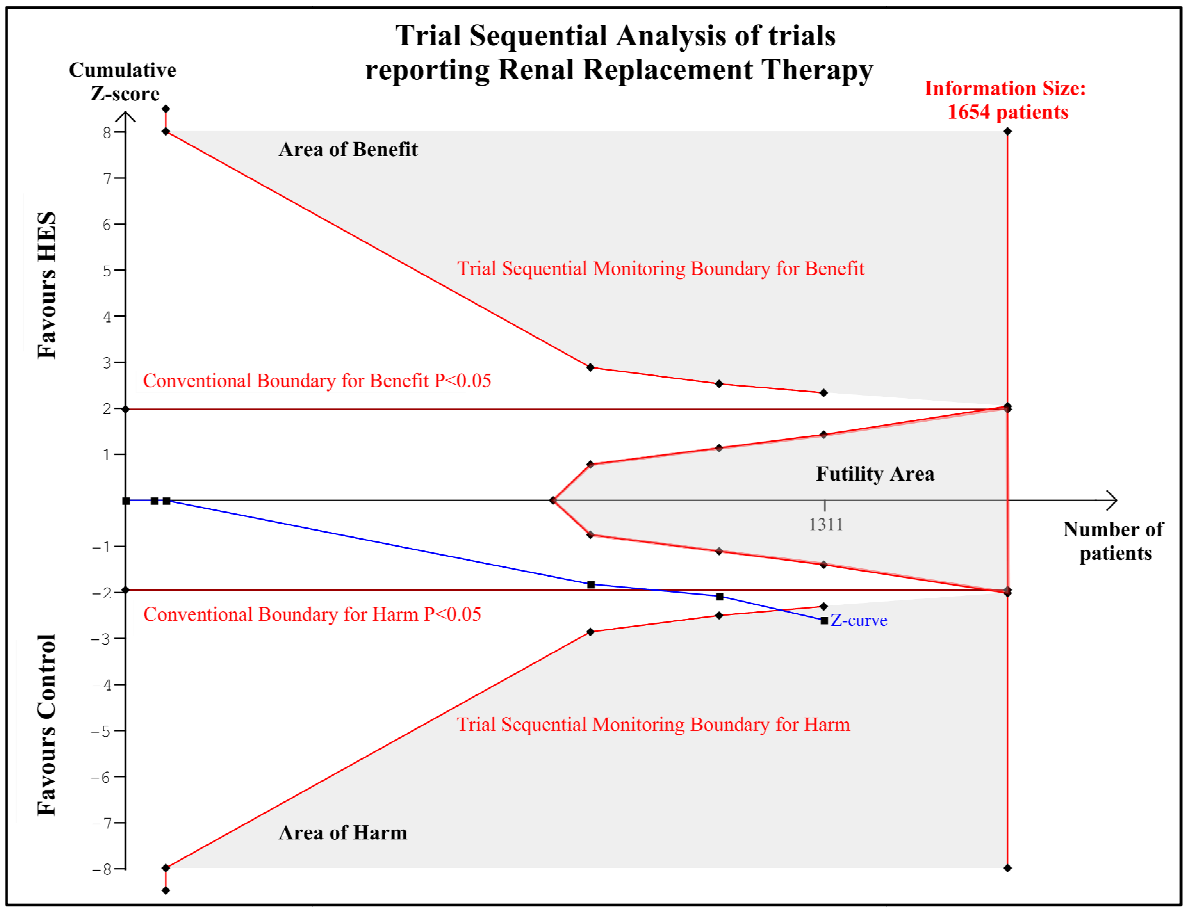

Figure 8 Trial Sequential Analysis of Renal Replacement Therapy. The required information size was not reached, but the blue z-curve crossed the trial sequential monitoring boundary for harm, and the adjusted confidence interval was 1.03 to 1.80 providing firm evidence of increased use of renal replacement therapy in patients treated with HES compared with crystalloid or albumin.

Conclusion

In conventional meta-analysis, tetrastarch vs. crystalloid or albumin increased the use of renal replacement therapy, red blood cells and lead to more serious adverse events in patients with sepsis. It seems unlikely that tetrastarch provides overall clinical benefit for patients with sepsis.

DISCUSSION

Principal findings

In the randomised, blinded 6S trial of patients with severe sepsis, HES 130/0.42 vs. Ringer's acetate increased the risk of death by 17%. The trial did not provide detailed information on cause of death, but patients treated with HES were more likely to have renal replacement therapy and bleedings both of which associated with mortality.

In the systematic review, tetrastarch vs. crystalloid or albumin lead to increased use of renal replacement therapy, red blood cells and serious adverse events in patients with sepsis. There was no overall mortality difference, but trials with low risk of bias suggested 11% increased risk of death with tetrastarch. After adjusting the results with trial sequential analysis signals for harm persisted.

Limitations and strengths - The 6S trial

Pragmatic trial design

The 6S trial was a state-of-the-art clinical trial with centralised randomisation, concealed allocation of trial fluid assignment and blinding of patients, clinical personnel, outcome assessors and statisticians all of which reduced the risk of bias [30, 32].

Pragmatic trials are distinguished from explanatory clinical trials, which are usually performed at earlier stages of drug development and aim at describing the biological effects of certain interventions. However, explanatory trials may not capture all adverse effects, and their results may not be applicable to other patient categories or to the daily clinical practice. In contrast, pragmatic trials are conducted at later stages and aim to answer common practical questions such as evaluating the risks and benefits in a broader range of patients in daily clinical practice. Pragmatic trial protocols need to be relative simple, which should not be misinterpreted as being less controlled, in order to include a high number of patients [33]. Thus, being a pragmatic trial the 6S trial was able to detect relatively small intervention effects and obtain results with a high external validity, but the trial delivered limited data explaining the mechanisms behind the results.

Patient selection

Investigating tetrastarch in patients with severe sepsis was a natural choice as adverse effects were seen in these patients with the former types of HES [3]. At the same time, patients with

sepsis are among the sickest ICU patients, and they might have the largest benefit of efficient fluid resuscitation with HES.

Randomised clinical trials in critical care tend to examine patients with relatively few comorbidities, who may not be representative for critically ill patients in general [34]. To avoid such patient selection bias, we had few exclusion criteria allowing for randomisation of two-thirds of the eligible patients. In addition, in Denmark and Norway contributing with 96% of the patients, informed consent could be obtained from two independent doctors, which allowed for fast inclusion of the sickest patients and probably explained the relatively high overall 90-day mortality of 47% in our trial. The fraction of included patients was higher than in many other ICU trials [3, 35, 36], but the ratio between eligible and randomised patients varied among our trial sites probably as a result of different patient populations, local problems in obtaining informed consent and incomplete registration of patients, who were never randomised. We did not demand a complete registration of all patients in the participating ICUs during the entire trial period nor did we systematically register patients with sepsis and no organ failure, which may also explain the high ratio of included patients.

Limitations regarding the patient population have been emphasised by other authors; the first being that the inclusion of patients with acute kidney injury conflicted with both clinical practice and the summary of product characteristics [37, unpublished manuscript seen for peer-review]. However, patients presented with various degree of acute kidney injury, and in agreement with the authorities and B Braun Medical manufacturing HES we excluded only patients receiving dialysis treatment at time of randomisation. This was supported by a survey of clinical practice in Scandinavian ICUs showing that acute kidney injury was a contraindication for tetrastarch in few ICUs only, while other ICUs considered acute kidney injury a specific indication for tetrastarch [26].

Secondly, the included patients might already have been fully resuscitated, as we did not have specific markers for hypovolemia among our criteria for inclusion, and as mean central venous pressure and lactate in the entire cohort were within the normal ranges [38–41]. In post-hoc analyses stratifying patients according to circulatory parameters and fluid given prior to randomisation, we were unable to confirm that the inclusion of fully resuscitated patients influenced the results (unpublished), but we did not have statistical power to fully assess this issue.

Finally, the included patients represented a heterogeneous population with regard to onset of disease, focus of infection, cardiovascular function etc., and our trial could not detect whether certain subgroups of patients experienced benefit with HES.

Intervention

We aimed at testing tetrastarch as used in clinical practice. Thus, our pragmatic protocol let the ICU clinicians themselves define thresholds for fluid therapy and goals for resuscitation, as all ICU clinicians are very experienced with hemodynamic treatment of septic patients. Consequently, the results of the 6S trial reflected the average effects of tetrastarch across different resuscitation algorithms, which likely increased the external validity of the results. The limitation was that we were unable to detect whether certain modes of administration were beneficial and others harmful. Unfortunately, people often misinterpret this pragmatic approach stating that the patients were inappropriately treated with no regard to their clinical condition [38–42].

Overdosing of HES hampered the interpretation of a previous HES trial [3, 43]. In our trial, 9% of the patients received more trial fluid than protocolised, but because the protocolised daily dose of HES was reduced, only two patients received HES at a dose higher than the recommended maximum daily dose of 50 ml/kg. Furthermore, the cumulative dose of HES remained below one daily maximum dose in the majority (54%) of patients.

Co-interventions

The stratified randomisation according to admission to a university hospital or not, the blinded design and inclusion of a relatively large number of patients were meant to balance co-interventions between the groups. However, as we did not assess all co-interventions during the trial period, we cannot exclude that differences in the use of co-interventions confounded the results.

Outcomes

Composite outcomes can be advantageous as several relatively rare events can be transformed into one more common composite outcome, which increases statistical power and lower the required sample size of a trial. However, the interpretation of group differences may be difficult as each component may contribute differently to the composite outcome, and the components may even point in different directions [44]. In our trial, interpretation of the primary outcome, death or dialysis-dependency at 90 days, was much easier as only mortality contributed to the group difference, but in future trials we would probably prefer using mortality alone.

It was a major strength that our trial had power to inform on mortality at 90 days. Even though the importance of mortality is indisputable, its validity is susceptible to the time of measurement. The 6S trial and our systematic review indicated that mortality should be measured after more than 28 days to fully show the intervention effect, but this may vary with patient category and type of intervention. On the other hand, mortality should not be measured solely too long after the intervention, because mortality in two groups will always converge to 100% and, thus, intervention effects will diminish over time.

Our secondary outcomes had several limitations. Outcomes such as doubling of creatinine, severe bleeding (intracranial bleeding or bleeding with concomitant transfusion of 3 units of red blood cells) and renal replacement therapy were prone to variation among doctors in use of fluid and blood products as well as in threshold for initiation of renal replacement therapy. The secondary outcomes were surrogates and should be interpreted with caution, as surrogate outcomes may not always associate with patient-important outcomes such as death, disability or long term quality of life. Moreover, the use of surrogate outcomes may lead to overestimation of the true intervention effect [45]. Having said that, our most important secondary outcome, renal replacement therapy, is widely acknowledged as a relatively robust surrogate outcome, since it closely associated with mortality in several large observational studies [46, 47].

We were able to track most patients through central registries. Consequently, we had 100% follow-up at 90 days, which is relatively seldom achieved in similar trials and a considerable strength, as this eliminates bias from dropout.

Statistics

Statistical analyses in paper I was done according to a pre-published statistical analysis plan [28], which was a major strength. In our primary analysis we tested for group difference in the primary outcome using a chi-square test. A logistic regression adjusted for the stratification variables had probably been a better choice, because the stratified randomisation correlated patients in the same stratum and ignoring this correlation may lead to too wide 95% confidence intervals and a reduction in statistical power [48, 49]. In our trial, however, the choice of analysis did not considerably affect the results (HES vs. Ringer's acetate on 90 day mortality: chi-square: RR 1.17, 95%-CI 1.01-1.36, P=0.034; logistic regression adjusted for stratification variables OR 1.37, 95%-CI 1.03-1.81, P=0.030).

As sensitivity analysis, we adjusted the analysis of the primary outcome for known risk factors for death. Adjusted analyses are more prone to bias, and some would argue that they should be omitted [50].

Analysis of all randomised patients according to their original group assignment - intention-to-treat analysis - is the golden standard for the analysis of randomised clinical trials. However, trials in the acute setting may be special as the narrow time frame may result in errors when evaluating the criteria for in- and exclusion, and patients may not receive the intervention either because the patients die quickly or the patients' condition change to the better e.g. fluid therapy may no longer be needed after conversion of an atrial flutter [51]. On one hand, these situations occur independently of group assignment, and the patients should be removed from the analysis, because including the patients may reduce group differences. On the other hand, the patients must remain in the analysis as they contribute to the equal distribution of baseline variables between the groups. We decided *a priori* to analyse a modified-intention-to-treat population [51], where we removed patients from the analysis who never received the intervention and who did not fulfil criteria for in- and exclusion. This resulted in the removal of two patients.

The use of delayed informed consent and informed consent by proxy also increased the risk of post-randomisation exclusions as sometimes patients and relatives either stop the ongoing trial or deny use of data. This may lead to loss of statistical power, shortened intervention with subsequent reduction of group differences, systematic errors as these dropouts may be related to outcome and eventually affect the results and conclusion of the trial [52]. In our trial, the intervention was stopped prematurely in 28 patients upon request, but only two patients denied use of data. Finally, the acute setting resulted in randomisation of two patients without proper informed consent. These two patients were removed from the database as well. Overall, six patients were excluded from our analysis, and the impact on the results of removing these patients was likely very small.

Reporting burdens to the patient and society in terms of length of dialysis, length of ventilator treatment and hospital length of stay is difficult as these variables are prone to survival bias. We reported these variables as "days alive and off dialysis", "days alive and off the ventilator" and "days alive and out of hospital", but it remains unclear whether the observed differences in hospital length of stay and dialysis treatment were true or simply caused by the increased mortality in the HES group. Alternative methods exist for reporting these outcomes in combination with mortality, but they are not yet widely accepted [53, 54].

Despite multiple testing for differences between the intervention groups we did not correct the P values accordingly, as certain outcomes may be correlated making exact correction of P values difficult [55]. Therefore, P values close to 0.05 in analyses of secondary and post-hoc outcomes should be interpreted with caution. In addition, the post-hoc analyses of paper II were not predefined, but the association between the use of HES and bleeding was relatively strong and in alignment with the results of other studies, which suggest a true finding.

Missing data was mainly an issue in the adjusted analyses, which were hampered by several patients with missed baseline covariates especially missing Simplified Acute Physiology Score (SAPS), a composite score based on 17 patient characteristics [56]. To properly assess this missingness, we made two adjusted analyses in paper I. In the first analysis, patients in the HES group were given the highest possible SAPS, and patients in the Ringer's acetate group were given the lowest possible SAPS. The second analysis was done vice versa. The advantage of this method is that the true intervention effect lies between these two worst-best case scenarios. The limitation is that the results of each scenario may be far apart. Therefore, in paper II we used multiple imputation of the missing covariates, which is considered golden standard for handling missing data [57, 58]. This method calculates the likely distributions of the missing variables from the other variables available, creates several datasets with imputations from these distributions, analyses each dataset separately and pools the results into one estimate of the true intervention effect.

Limitations and strengths - The Systematic Review

Design

The main strength of our systematic review was the compliance with the recommendations of the Cochrane Collaboration [30] and reporting according to the PRISMA guidelines [59]. This included a pre-published protocol, an up to date extensive literature search with no language restrictions, independent screening of all references by two authors, inclusion of trials irrespective of publication, language status and reported outcomes, independent data extraction by two authors, bias risk assessment and contact with the corresponding authors of the included trials for additional information.

We restricted our review to trials investigating tetrastarch in patients with sepsis and excluded consequently several trials investigating the former starches and/or patients without sepsis. We did this based on the anticipation that the former starches were no longer used in

clinical practice, and that tetrastarch potentially had different effects in patients with sepsis, who were sicker than e.g. patients undergoing elective surgery. In addition, we expected that most available data would be from patients with sepsis, which would prevent us from drawing conclusions regarding the effects of tetrastarch in patients without sepsis anyway. The advantage of this approach was a limited work load and a lower risk of comparing apples and oranges. Limitations were loss of power, increased risk of type II errors and the inability to confirm or reject the hypothesis that the clinical effects vary among different kinds of HES solutions and different patient categories. Moreover, a narrow scope allows authors, who wish to verify a desired hypothesis, to define specific criteria for inclusion according to their pre-existing knowledge of trials with certain outcomes [30]. This may have been the case in a recent review of HES sponsored by a HES manufacturer, which included studies of healthy volunteers, but did not include trials of patients with sepsis [60].

Trial Sequential Analysis

Another strength of our review was the application of trial sequential analysis, because conventional meta-analyses may produce random errors due to sparse data and repetitive testing of accumulating data [61, 62]. In trial sequential analysis, the number of patients needed to show or reject a specific intervention effect, the required information size, is calculated and then used to evaluate the strength of the P value of the conventional meta-analysis. This approach is similar to sample size estimation and interim analysis of a single trial. The required information size is estimated from 1) the risk of type I and type II errors (usually 5% and 20%, respectively), 2) the size of the intervention effect and 3) the event proportion. This estimation is not straightforward as the size of the intervention effect may be selected among 1) the *a priori* anticipated effect, 2) the observed overall intervention effect in the meta-analysis or 3) the observed intervention effect in trials with lowest risk of bias. Similarly, the event proportion may be either the anticipated or observed, and finally the required information size should be adjusted for heterogeneity among trials, which may be the *a priori* anticipated heterogeneity or the observed heterogeneity in the meta-analysis.

Consequently, the required information size and the result of the trial sequential analysis will depend on the selected parameters, which is the main limitation of the analysis. No exact recommendation for this selection can be made as anticipated values from similar clinical settings may be imprecise, and observed values may be biased if only few small trials exist or if the

available data originates from one very large trial only [61, 63–65]. To increase the robustness of our results we pre-specified the choice of parameters in our review protocol.

Current evidence for the use of HES

Retraction of HES studies by Joachim Boldt

The German professor Joachim Boldt was world renowned for his many trials of HES mainly in the surgical setting. In 2011, 88 of his 102 papers published since 1999 were retracted due to failure of acquiring ethical approval for research and fabrication of study data [66, 67]. These papers constituted a major part of the clinical data supporting the use of HES, and after the retraction recommendations against its general use in the ICU setting were issued [68].

Broad systematic reviews

Following the retraction of the papers by Boldt and the publication of the 6S and other recent trials several updated meta-analyses were published [69–71].

Perel et al. pooled the data of trials comparing any kind of HES solution vs. crystalloid in critically ill patients and found a statistical significant increased risk of death with HES (relative risk 1.10, 95%-CI 1.02-1.19, P=0.02).

Zarychanski et al. compared any kind of HES solution with crystalloid, albumin or gelatine in critically ill patients. The authors found a relative risk of death of 1.07 with HES, which became significant after the exclusion of non-retracted papers by Joachim Boldt. In addition, HES significantly increased the risk of having renal replacement therapy (relative risk 1.32, 95%-CI 1.15-1.50, P<0.001).

Gattas et al. compared tetrastarch vs. any type of control fluid for resuscitation of acutely ill patients. The pooled relative risk of death and renal replacement therapy were 1.08 (95%-CI 1.00-1.17, P=0.05) and 1.25 (95%-CI 1.08-1.44, P=0.002), respectively.

The findings regarding mortality and renal replacement therapy in these reviews were comparable to those of our systematic review. Thus, the conclusions of our systematic review will not be fundamentally changed by adding data from trials of other kinds of HES, other comparator fluids and other critically ill patients.

However, the meta-analyses cannot rule out that certain subgroups of patients may benefit from HES, because ICU trials contributed with the majority of patients. Consequently, the current evidence for the use of HES in various subgroups is reviewed in the following sections.

HES in sepsis

In the Efficacy of Volume Substitution and Insulin Therapy in Severe Sepsis (VISEP) trial patients with severe sepsis in the ICU were randomised to open-labelled fluid resuscitation with 10% HES 200/0.5 vs. Ringer's lactate [3]. The trial was stopped by its data and safety monitoring board after the inclusion of 537 patients due to significantly greater incidence of acute kidney failure (35% vs. 23%, P=0.002) and use of renal replacement therapy (31% vs. 19%, P=0.001) as well as a trend towards increased risk of death at 90 days (41% vs. 34%, P=0.09) in the HES group. The ratio between the hypertonic HES used in this trial and Ringer's lactate was 1.3, and the use of red blood cells was higher in the HES group.

Despite using a different HES solution at a higher dose and having an open-labelled design, the results of the VISEP trial were strikingly similar to those of the 6S trial with regard to mortality, survival curves, renal impairment and use of blood products, and also they were in line with the results of our systematic review. Together with the results of the 6S trial and our systematic review, the VISEP trial provides evidence that HES should not be used in patients with sepsis and indicates that harmful clinical effects with HES in sepsis may be a class-effect independent of type of HES solution.

HES in general ICU patients

The Crystalloid vs. Hydroxyethyl Starch Trial (CHEST) was finished few months after the 6S trial [36]. In this high-quality pragmatic trial 7,000 general ICU patients were randomised to fluid resuscitation with either HES 130/0.4 or normal saline. Overall, fluid doses were two to three times lower than those in the 6S trial, but the patients in the HES group received less trial fluid (526 ±425 vs. 616±488 ml per day in the first four days), had higher central venous pressure (CVP) and fewer patients developed new circulatory failure (36.5% vs. 39.9%, P=0.03). However, this did not result in clinical benefit regarding patient-important outcomes as more patients in the HES group received renal replacement therapy (7.0% vs. 5.8%, P=0.04) and more had pruritus (4.0 % vs. 2.2%, P<0.001). 90-day mortality did not differ significantly between the groups neither in the whole population (18% vs. 17%, P=0.26) nor in the predefined subgroup of patients with sepsis, but the estimates favoured saline.

The limitations of this trial were the inclusion of elective surgical patients and exclusion of patients considered unlikely to survive, which resulted in lower mortality than expected and inadequate statistical power to detect small mortality differences. However, the trial showed that

adverse renal effects of HES are not limited to patients with sepsis, but is also seen in a broader group of critically ill patients.

The *Colloids Compared to Crystalloids in Fluid Resuscitation of Critically Ill Patients* (CRISTAL) trial randomised 3,000 critically ill ICU patients to open-labelled resuscitation with HES, albumin or any other colloid vs. any crystalloid solution, but is not yet published [72]. The open-label design and the use of different colloids in the colloid arm make direct conclusions regarding the effect of HES very difficult.

HES in trauma

Tetrastarch has been investigated in one trauma trial only, which randomised 115 severely injured trauma patients to masked resuscitation with HES 130/0.4 vs. normal saline [73]. The reporting of this trial has been heavily criticised, because most analyses were of subgroups and outcomes that were not predefined, which made it unclear how HES vs. saline really affected the patients in this trial [74, 75]. However, the trial suggested a small volume sparring effect, impaired coagulation and increased use of blood products with HES. The trial could not adequately assess safety outcomes, but 30-day mortality estimates favoured saline (22% vs. 12%, RR 1.83, 95%-CI 0.79-4.24, p=0.15 (data obtained from the CONSORT diagram and [74]).

Two other trials using older starches in trauma did not provide evidence for safe use of HES in these patients [76, 77].

HES peri- and post-operatively

Trials in surgery show divergent results with regard to benefits and harms with HES, which may be due to varying populations and varying dose regimens. In addition, most trials in surgery have poor design such as small sample sizes, lack of blinding, no allocation concealment and limited follow-up time all of which increase the risk of erroneous results [24, 71].

The volume effect of HES in surgery is poorly investigated, because only few trials were left comparing the potency of tetrastarch vs. crystalloid after the retraction of the studies by Boldt. Overall, the volume effect of 1 litre of tetrastarch seems equalled by 1 to 1.5 litre of crystalloid in surgical patients [78-80], which is comparable to findings in critically ill patients [36, paper I, paper III].

Haemostatic impairment is probably the largest concern with HES in surgical patients. In a meta-analysis of patients undergoing cardiac surgery HES vs. albumin increased postoperative bleeding, doubled the risk of reoperation for bleeding (RR 2.24, 95%-CI 1.14 to 4.40, P=0.02) and

significantly increased transfusion with red blood cells, fresh frozen plasma and platelets [81]. However, none of the included trials used tetrastarch so these results may apply to older starches only. Supporting this, a systematic review sponsored by the HES manufacturer Fresenius Kabi found lower blood loss, drainage loss and transfused volume of red blood cells with tetrastarch vs. HES 200/0.5 [82].

Even though tetrastarch may result in less coagulation impairment than former HES solutions, signs of haemostatic impairment with tetrastarch vs. crystalloid or albumin have been observed in several trials including prolonged activated partial thromboplastin time (APTT) [83], prolonged prothrombin time [84] and impaired thrombelastometric parameters [83–87], but only one trial reported significantly increased blood loss during surgery [88]. The clinical relevance of these findings are uncertain, but the association between bleeding and mortality in the 6S trial indicate that HES induced coagulopathy may affect patient outcome.

Renal impairment and mortality were rare events in the surgical trials, because they were mainly conducted in elective patients, and neither single trials nor meta-analyses had the statistical power to adequately assess renal function or mortality in these patients. Thus, these very important safety issues of HES have not been adequately assessed in surgery.

Mechanisms behind adverse effects with HES

It is difficult to identify the exact mechanisms behind the increased mortality observed in the 6S trial and suggested by systematic reviews as pragmatic trials deliver limited data on mechanisms behind intervention effects. Moreover, the cause of death is difficult to establish in ICU patients. In both the 6S and VISEP trials the separation of the survival curves occurred after several weeks indicating long-term adverse effects. However, the ability to sustain life in the ICU with e.g. vasopressors and renal replacement therapy allows for short-term effects followed by late death as well. The existence of some short-term effects is supported by post-hoc analyses of the 6S trial showing that the main group differences in use of renal replacement therapy and occurrence of bleeding happened in the first few days after randomisation (paper II and unpublished data).

A systematic review pooling the results of pharmacokinetic studies recently showed that almost half of the infused tetrastarch was deposited in the tissues 24 hours after infusion in healthy volunteers and elective surgical patients [10]. Once in the tissues, HES is taken up by several cell types and stored in the lysosomes, where it is resistant to degradation. In alignment with this, HES has been found in biopsies from several organs including the liver, kidney, skin, intestine, striated muscle, spleen and placenta up to several years after HES treatment [17–21].

How these deposits affect cell and organ function is less clear, but HES may induce osmotic cellular changes and damage [17]. Reports regarding a subsequent immune response are divergent as both anti- and pro-inflammatory properties with HES have been reported [89, 90]. Thus, both short- and long-term adverse effects may be explained by tissue deposition, but the clinical importance of tissue deposition and a causal pathway from deposition to harm are not yet fully elucidated.

The mechanisms behind HES induced coagulopathy are relatively well described *in vitro* and in surgery. First, hemostasis is affected by hemodilution, which may be more pronounced with HES than with crystalloids. Secondly, HES exerts an additive non-dilutional alteration mainly through reduced platelet function and clot strength as well as affected von Willebrand factor, factor XIII and fibrinogen/fibrin polymerisation [85–87, 91–93]. In line with this, HES has been found in the lysosomes of platelets in an unpublished study [17]. Unpublished data on a subgroup of 260 patients in the 6S trial show reduced maximum amplitude in thrombelastography after incubation with a platelet inhibitor, which probably reflects reduced fibrinogen/fibrin polymerisation and indicates that the mechanisms for coagulopathy in septic patients are similar to those in surgical patients. Since there is a high turnover of platelets and coagulation factors as well as a short half-life of tetrastarch in plasma, HES induced coagulopathy is likely to persist only for few days after infusion, which is in line with our findings in the post-hoc analyses of the 6S trial.

The clinical effect of HES solutions may depend on the plant origin as the C2:C6 pattern for hydroxyethylation is higher in maize-derived tetrastarch than in potato-derived tetrastarch, but neither clinical nor pre-clinical data provide evidence for this notion [91, 94].

Tetrastarch is claimed to have fewer side effects due to its lower molecular weight, lower substitution ratio and faster plasma clearance than older formulations of HES [8, 9], but interestingly the above pharmacokinetic review suggested that the faster clearance is mainly due to increased tissue deposition rather than increased elimination [10]. If this is true, side effects related to tissue deposition may be independent of type of HES solution, and this may explain why adverse events with HES seem to be a class effect.

Alternatively, the adverse effects observed with HES are due to concomitant harmful interventions. In most trials the use of HES leads to more use of blood products, which may have late adverse effects [95], but other concomitant interventions have not yet been identified.

CONCLUSION AND FUTURE PERSPECTIVES

The 6S trial is one of several high-quality clinical trials in critically ill patients with and without sepsis that now provide evidence that the use of tetrastarch impairs kidney function and hemostasis and may even increase mortality. At the same time, the circulatory benefits with tetrastarch, constituting the rationale for its use, seem much smaller than previously estimated.

Whether the findings in critically ill patients can be extrapolated to other types of patients is unclear, as data from these patients are limited and so far no group of patients with an overall benefit of HES beyond surrogate markers has been identified.

Based on the recent trial results, the European Medicines Agency's Pharmacovigilance Risk Assessment Committee has reviewed the risk-benefit balance of HES solutions and recommended as recent as mid-June that the marketing authorisations of all HES solutions should be suspended in the European Union [96]. Currently, this recommendation is reviewed by the European Medicines Agency and awaits a final decision. The U.S. Food and Drug Administration is conducting a similar review, but has not yet reached a decision. If HES remains on the market for use in certain types of patients, large pragmatic trials will urgently be needed to ensure their safety.

There is a lesson to be learned from the history of HES: Large, pragmatic trials with patient-important outcomes must be performed as part of drug development to confirm expectations from theory and smaller studies. Otherwise, we risk treating millions of patients with drugs that likely cause more harm than good, and who can live with that?

FUNDING AND CONFLICTS OF INTERESTS

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Paper I

ORIGINAL ARTICLE

Hydroxyethyl Starch 130/0.42 versus Ringer's Acetate in Severe Sepsis

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ABSTRACT

BACKGROUND

Hydroxyethyl starch (HES) is widely used for fluid resuscitation in intensive care units (ICUs), but its safety and efficacy have not been established in patients with severe sepsis.

METHODS

In this multicenter, parallel-group, blinded trial, we randomly assigned patients with severe sepsis to fluid resuscitation in the ICU with either 6% HES 130/0.42 (Tetraspan) or Ringer's acetate at a dose of up to 33 ml per kilogram of ideal body weight per day. The primary outcome measure was either death or end-stage kidney failure (dependence on dialysis) at 90 days after randomization.

RESULTS

Of the 804 patients who underwent randomization, 798 were included in the modified intention-to-treat population. The two intervention groups had similar baseline characteristics. At 90 days after randomization, 201 of 398 patients (51%) assigned to HES 130/0.42 had died, as compared with 172 of 400 patients (43%) assigned to Ringer's acetate (relative risk, 1.17; 95% confidence interval [CI], 1.01 to 1.36; P=0.03); 1 patient in each group had end-stage kidney failure. In the 90-day period, 87 patients (22%) assigned to HES 130/0.42 were treated with renal-replacement therapy versus 65 patients (16%) assigned to Ringer's acetate (relative risk, 1.35; 95% CI, 1.01 to 1.80; P=0.04), and 38 patients (10%) and 25 patients (6%), respectively, had severe bleeding (relative risk, 1.52; 95% CI, 0.94 to 2.48; P=0.09). The results were supported by multivariate analyses, with adjustment for known risk factors for death or acute kidney injury at baseline.

CONCLUSIONS

Patients with severe sepsis assigned to fluid resuscitation with HES 130/0.42 had an increased risk of death at day 90 and were more likely to require renal-replacement therapy, as compared with those receiving Ringer's acetate. (Funded by the Danish Research Council and others; 6S ClinicalTrials.gov number, NCT00962156.)

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*Members of the Scandinavian Starch for Severe Sepsis/Septic Shock (6S) trial group are listed in the Supplementary Appendix, available at NEJM.org.

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Intravenous fluids are the mainstay of treatment for patients with hypovolemia due to severe sepsis. Colloid solutions are used to obtain rapid and lasting circulatory stabilization, but there are limited data to support this practice.[1] The Surviving Sepsis Campaign guidelines recommend the use of either colloids or crystalloids,[2] but high-molecular-weight hydroxyethyl starch (HES) may cause acute kidney failure in patients with severe sepsis, as observed in two randomized trials.[3,4] Those trials had substantial limitations, and participants received HES solutions with a molecular weight of 200 kD and a substitution ratio (the number of hydroxyethyl groups per glucose molecule) of more than 0.4.[3,4] These solutions have largely been replaced by HES solutions with a lower molecular weight and a lower substitution ratio, HES 130/0.4.[5,6] There are limited data about the effects of HES 130/0.4 in patients with severe sepsis,[7] and its routine use has recently been discouraged.[8]

Given the lack of efficacy data and concerns about safety, we conducted the Scandinavian Starch for Severe Sepsis/Septic Shock (6S) trial to evaluate the effects of HES 130/0.4 as compared with Ringer's acetate on the composite outcome of death or end-stage kidney failure in patients with severe sepsis.

Methods

Trial Design and Oversight

Patients were screened and underwent randomization between December 23, 2009, and November 15, 2011, in Denmark, Norway, Finland, and Iceland after the appropriate approvals. Patients were screened at 26 general intensive care units (ICUs) in 13 university and 13 nonuniversity hospitals. Written informed consent was obtained from patients or their legal surrogates before enrollment. In all cases, consent was obtained from the patient when possible. If consent was withdrawn or not granted, we asked the patient or surrogate for permission to continue registration of trial data and to use these data in the analyses. The protocol, including details on trial conduct and procedures and the statistical analysis plan, has been published previously[9] and is available with the full text of this article at NEJM.org. B. Braun Medical provided trial fluids to all trial sites free of charge. Neither the funders nor B. Braun Medical had influence on the protocol, trial conduct, or data analyses or reporting. The steering committee vouches for the accuracy and completeness of the data and the analysis and the fidelity of the study to the protocol, and it made the decision to submit the manuscript for publication. The writing committee had full access to all data and wrote the manuscript with input from all authors. The trial was endorsed by the European Clinical Research Infrastructures Network.

This trial was an investigator-initiated, multicenter, blinded, stratified, parallel-group clinical trial with a computer-generated allocation sequence and centralized, blinded randomization. We randomly assigned patients with severe sepsis in a 1:1 ratio to fluid resuscitation with either HES 130/0.42 or Ringer's acetate. Treatment assignments were concealed from patients, clinicians, research staff, the data monitoring and safety committee, the statistician, and the writing committee when it wrote the first draft for the abstract (for details, see the Supplementary Appendix, available at NEJM.org). Randomization was stratified according to the presence or absence of shock, the presence or absence of active hematologic cancer, and admission to a university or nonuniversity hospital, because these characteristics might have influenced the outcome.[10,11] The conduct of the trial and the safety of the participants were overseen by the data monitoring and safety committee, which performed an interim analysis after 400 patients had undergone randomization.

Patients

We screened patients 18 years of age or older who needed fluid resuscitation in the ICU, as judged by the ICU clinicians, and who fulfilled the criteria for severe sepsis within the previous 24 hours[12] (for details, see the Supplementary Appendix). Patients were excluded for the reasons shown in Figure 1.

Interventions

Trial fluid (6% HES 130/0.42 in Ringer's acetate [Tetraspan 6%, B. Braun] or Ringer's acetate [Sterofundin ISO, B. Braun]; see the Supplementary Appendix for electrolyte content) was used when ICU clinicians judged that volume expansion was needed in the ICU for a maximum of 90 days. Trial fluid was delivered in identical bags (Ecobag, B. Braun), which were fully covered in custom-made black, opaque plastic bags and sealed by staff members who were not involved in data registration or patient care. The maximum daily dose

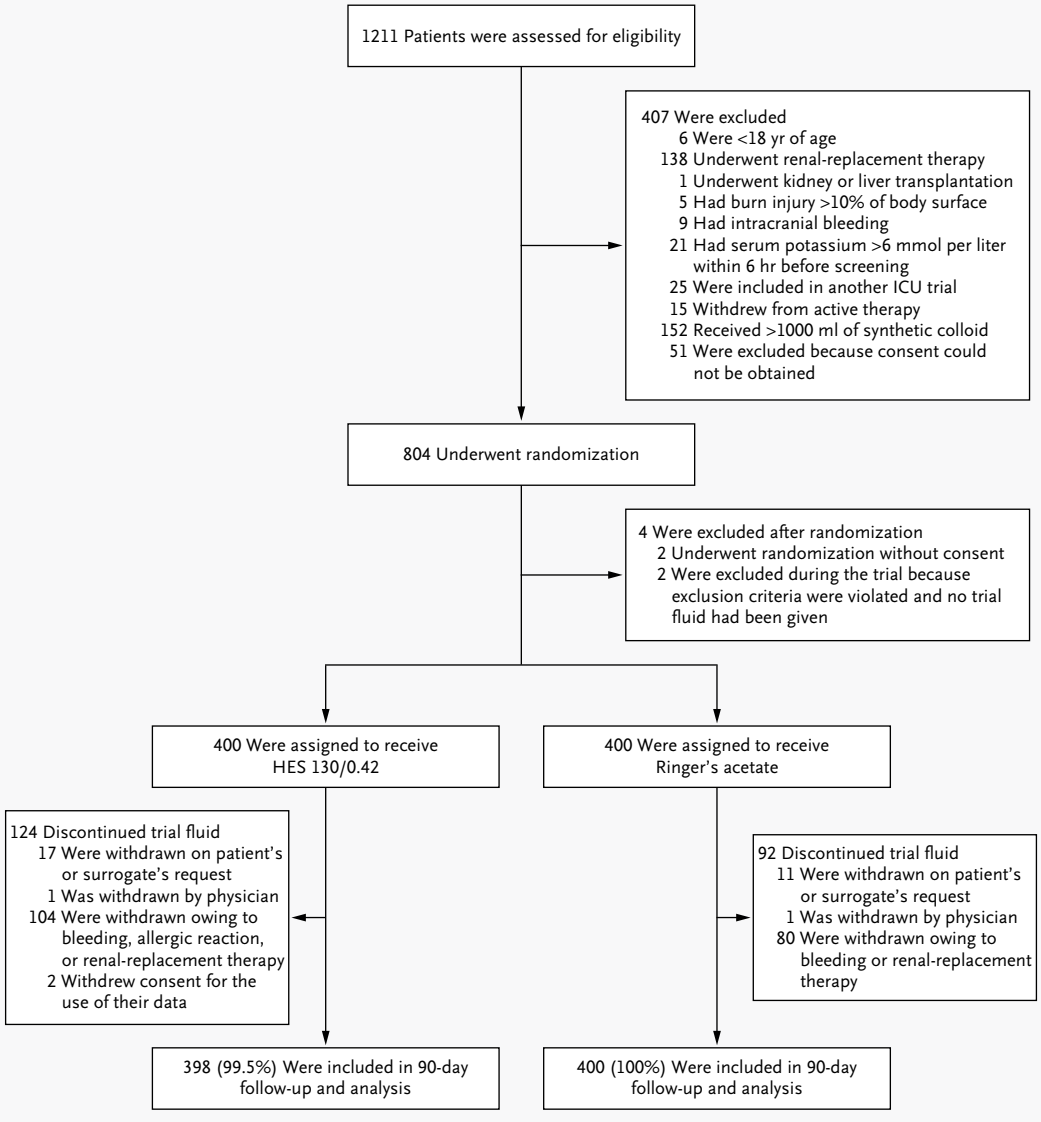


Figure 1. Randomization and Follow-up of Study Patients.

Patients were excluded for medical reasons or if they had previously undergone randomization; if they had received more than 1000 ml of synthetic colloid in the previous 24 hours; if they were enrolled in another intensive care unit (ICU) trial of drugs with effects on circulation, renal function, or coagulation; or if consent could not be obtained. Sixteen patients met two exclusion criteria. Two patients were excluded after they had been randomly assigned to a treatment group because consent had not been obtained before randomization. Another two patients were excluded, as specified by the statistical analysis plan, because subsequent assessment showed that they met exclusion criteria and they never received trial fluid. Thus, four additional patients were randomly assigned to a study group to obtain the full sample size. Two patients withdrew consent for the use of their data after the end of the trial. HES denotes hydroxyethyl starch.

was 33 ml per kilogram of ideal body weight (for details, see the Supplementary Appendix). If doses higher than the maximum daily dose were required, unmasked Ringer's acetate was used, regardless of the treatment assignment. In the event of severe bleeding, a severe allergic reaction, or the commencement of renal-replacement therapy for acute kidney injury, trial fluid was permanently stopped and 0.9% saline or Ringer's lactate was given for volume expansion in the ICU until

90 days after randomization. All other interventions were at the discretion of the ICU clinicians, and crystalloid and albumin solutions were allowed for indications other than volume expansion. Criteria for renal-replacement therapy were not included in the protocol.

OUTCOMES

The composite primary outcome was death or dependence on dialysis 90 days after randomization[13]; the latter was defined as the use of any renal-replacement therapy during the period from 86 to 94 days after randomization. In addition, these outcomes were analyzed separately. Secondary outcomes were death at 28 days; death at the time of the latest follow-up assessment; severe bleeding (defined as clinical bleeding that required 3 or more units of packed red cells within 24 hours)[14] while the patient was in the ICU; severe allergic reactions; the score on the Sepsis-related Organ Failure Assessment (SOFA), modified by excluding the Glasgow Coma Scale (Table S9 in the Supplementary Appendix),[15] at day 5 after randomization (the SOFA score includes subscores ranging from 0 to 4 for each of five components [circulation, lungs, liver, kidneys, and coagulation], with higher scores indicating more severe organ failure); the development of acute kidney injury (use of renal-replacement therapy or a renal SOFA score of 3 or higher after the patient had a renal SOFA score of 2 or lower at randomization) in the ICU after randomization; doubling of the plasma creatinine level in the ICU after randomization[3,4]; acidosis (arterial pH <7.35) in the ICU; and percentages of days alive without renal-replacement therapy, days alive without mechanical ventilation, and days alive out of the hospital in the 90 days after randomization.

Data for the outcome measures were obtained by the 6S trial investigators or their delegates from patient files, national registries, and telephone contact with patients and hospitals for the 90-day follow-up period (not limited to the index admission). The final mortality follow-up was conducted on February 16, 2012, which was 90 days after randomization of the last patient.

STATISTICAL ANALYSIS

We calculated that we would need to enroll 800 patients for the study to have 80% power to show an absolute between-group difference of 10 percentage points in the primary outcome measure at a two-sided alpha level of 0.05, assuming a 45% mortality rate[6,16] and a 5% rate of dependence on dialysis at 90 days.[17,18] During the trial, four patients were excluded after randomization (two for whom consent had not been obtained and two who met exclusion criteria and never received trial fluid). Four additional patients were randomly assigned to a study group to obtain the full sample (Fig. 1).[19]

All analyses were performed by one of the authors before the breaking of the randomization code, according to International Conference on Harmonization–Good Clinical Practice guidelines[20] and the statistical analysis plan. The analyses were performed on data from the modified intention-to-treat population, defined as all randomly assigned patients except those who could be excluded without the risk of bias (four patients who underwent randomization by mistake and who never received trial fluid)[19] and those for whom we did not have consent for the use of data (two patients) (Fig. 1). In the per-protocol analyses, patients with one or more major protocol violations were excluded; see the Supplementary Appendix for definitions of the trial populations.

Data were analyzed with the use of unadjusted chi-square tests for binary outcome measures and Wilcoxon signed-rank tests for rate and ordinal data. We also compared the primary outcome in the per-protocol populations and in the predefined subgroups (patients with shock or acute kidney injury at the time of randomization) and used multiple logistic-regression analyses in the modified intention-to-treat population to adjust for differences in baseline variables, including known risk factors for death or acute kidney injury. Details on the handling of missing data are given in the Supplementary Appendix. All analyses were performed with the use of SAS software, version 9.3. A two-sided P value of less than 0.05 was considered to indicate statistical significance.

RESULTS

STUDY POPULATION

The 798 patients — 398 in the HES 130/0.42 group (hereafter called the starch group) and 400 in the Ringer's acetate group (Fig. 1) — were followed for at least 90 days and analyzed in the group to which they were assigned. Baseline characteristics were similar in the two groups (Table 1, and Table S1 in the Supplementary Appendix).

Table 1. Baseline Characteristics of the Patients.*

Characteristic	HES 130/0.42 (N=398)	Ringer's Acetate (N=400)
Age — yr		
Median	66	67
Interquartile range	56–75	56–76
Male sex — no. (%)	239 (60)	244 (61)
Ideal body weight — kg†		
Median	72	72
Interquartile range	60–80	60–80
Admitted to university hospital — no. (%)	194 (49)	188 (47)
Surgery — no. (%)‡		
Emergency	114 (29)	116 (29)
Elective	34 (9)	48 (12)
Source of ICU admission — no. (%)		
Emergency department	109 (27)	94 (24)
General ward	177 (44)	196 (49)
Operating or recovery room	59 (15)	54 (14)
Other ICU in the same hospital	21 (5)	14 (4)
Other hospital	32 (8)	42 (10)
Source of sepsis — no. (%)§		
Lungs	212 (53)	229 (57)
Abdomen	130 (33)	133 (33)
Urinary tract	56 (14)	50 (12)
Soft tissue	38 (10)	46 (12)
Other	43 (11)	33 (8)
SAPS II — median (interquartile range)¶	50 (40–60)	51 (39–62)
SOFA score — median (interquartile range)‖	7 (5–9)	7 (5–9)
Shock — no. (%)**	336 (84)	337 (84)
Acute kidney injury — no. (%)††	142 (36)	140 (35)
Mechanical ventilation — no. (%)	240 (60)	245 (61)

* None of the differences between the two groups were significant (P>0.05). The values for the Simplified Acute Physiology Score (SAPS)[21] II, Sepsis-related Organ Failure Assessment (SOFA)[15] score, acute kidney injury, and mechanical ventilation (invasive or noninvasive) pertain to the 24 hours before randomization. For additional baseline characteristics, see Table S1 in the Supplementary Appendix. HES denotes hydroxyethyl starch, and ICU intensive care unit.

† Ideal body weight was calculated as estimated height in centimeters minus 100 for men and estimated height in centimeters minus 105 for women.

‡ Data are shown for patients who underwent surgery during the index hospitalization but before randomization.

§ Some patients had more than one source of infection. The "other" category included sepsis from a vascular catheter–related infection, meningitis, or endocarditis, as well as sepsis from unknown sources.

¶ SAPS II is calculated from 17 variables; scores range from 0 to 163, with higher scores indicating more severe disease. Data regarding 1 or 2 of the 17 variables were missing for 105 patients in the HES 130/0.42 group and 108 patients in the Ringer's acetate group, so the scores for these patients are not included here.

‖ The SOFA score includes subscores ranging from 0 to 4 for each of five components (circulation, lungs, liver, kidneys, and coagulation). Aggregated scores range from 0 to 20, with higher scores indicating more severe organ failure (Table S9 in the Supplementary Appendix). The scoring was modified because cerebral failure was not assessed. One of the five subscores was missing for two patients in the HES 130/0.42 group, so their scores are not included here.

** Shock at randomization was defined as a mean arterial pressure of less than 70 mm Hg, the need for ongoing treatment with vasopressor or inotropic agents, or a plasma lactate level of more than 4.0 mmol per liter in the hour before randomization.

†† Acute kidney injury was defined as a renal SOFA score of 2 or higher (plasma creatinine level >1.9 mg per deciliter [170 μmol per liter] or urinary output <500 ml per day).

FLUID THERAPY, USE OF BLOOD PRODUCTS, AND CIRCULATORY EFFECTS

Of the 798 patients, 779 (98%) received trial fluid. The median cumulative volume of fluid received was 3000 ml (interquartile range, 1507 to 5100) in the starch group and 3000 ml (interquartile range, 2000 to 5750) in the Ringer's acetate group (P=0.20), equaling 44 ml per kilogram of ideal body weight (interquartile range, 24 to 75) and 47 ml per kilogram (interquartile range, 25 to 76), respectively (P=0.18). Seventy-seven patients (39 in the starch group and 38 in the Ringer's acetate group) received open-label synthetic colloids in the ICU during the 90-day trial period. Sixty-nine patients (28 in the starch group and 41 in the Ringer's acetate group) received trial fluid at doses higher than the protocol-specified maximum daily dose. Only 2 patients in the starch group received HES 130/0.42 at a dose higher than the maximum daily dose recommended by the manufacturer (50 ml per kilogram). Details on other fluid volumes and balances and protocol violations are provided in Table 2 and in the Supplementary Appendix, including Tables S2 and S3.

More patients in the starch group than in the Ringer's acetate group received blood products (relative risk, 1.20; 95% confidence interval [CI], 1.07 to 1.36; P=0.002), including packed red cells (relative risk, 1.28; 95% CI, 1.12 to 1.47; P<0.001) (Table 2, and Table S2 in the Supplementary Appendix). There were no significant differences between the two groups in the circulatory variables assessed at baseline and during the 24 hours after randomization (Table S4 in the Supplementary Appendix).

OUTCOMES

The primary outcome, death or dependence on dialysis at 90 days after randomization, occurred in 202 patients (51%) in the starch group as compared with 173 patients (43%) in the Ringer's acetate group (relative risk, 1.17; 95% CI, 1.01 to 1.36; P=0.03). One patient in each group was dependent on dialysis at day 90 (Table 3). Similar results were obtained in the multiple logistic-regression and per-protocol analyses (see the Supplementary Appendix, including Table S6). The survival curves for the two intervention groups are shown in Figure 2, and Figure S1 in the Supplementary Appendix. The two predefined subgroup analyses showed no heterogeneity in the effect of HES 130/0.42 on the primary outcome in patients with shock or acute kidney injury at the time of randomization (Fig. 2).

More patients in the starch group than in the Ringer's acetate group received renal-replacement therapy (Table 3). Among all patients, renal-replacement therapy was associated with increased 90-day mortality (61%, vs. 44% for those not receiving renal-replacement therapy; P<0.001). In the starch group, 38 patients (10%) had severe bleeding, as compared with 25 (6%) in the Ringer's acetate group (relative risk, 1.52; 95% CI, 0.94 to 2.48; P=0.09) (Table 3).

The percentage of days alive without renal-replacement therapy and the percentage of days alive and out of the hospital were lower in the starch group than in the Ringer's acetate group (Table 3). None of the remaining secondary outcomes differed significantly between the groups (Table 3), but some of the post hoc analyses of kidney injury and bleeding showed significant differences (Tables S7 and S8 in the Supplementary Appendix).

DISCUSSION

In this international, blinded, randomized trial of fluid resuscitation of patients with severe sepsis, HES 130/0.42 significantly increased the risk of death or dependence on dialysis at day 90, as compared with Ringer's acetate. The difference was due to an increased risk of death at 90 days, because only 1 patient in each group was dependent on dialysis at 90 days. HES 130/0.42 increased the absolute risk of death at 90 days by 8 percentage points, corresponding to a number needed to harm of 13. Similar results were observed in analyses adjusted for risk factors and in the subgroups of patients with shock or acute kidney injury at the time of randomization.

The increased risk of death observed with HES 130/0.42 in our trial is similar to that observed in the Efficacy of Volume Substitution and Insulin Therapy in Severe Sepsis (VISEP) trial with HES 200/0.5,[4] but that trial was not powered to show the difference with statistical significance. The separation of the survival curves occurred around day 20 in both trials, indicating late deaths induced by HES. Both trials showed that HES was associated with impaired kidney function and increased use of renal-replacement therapy, the negative consequences of which are well known and were confirmed by our data.[17,22] In both trials,

Table 2. Fluid Therapy before and after Randomization.*

Variable	HES 130/0.42 (N=398)			Ringer's Acetate (N=400)			P Value†
	Patients	Volume Received‡		Patients	Volume Received‡		
		median	interquartile range		median	interquartile range	
	no./total no.§	*ml*		*no./total no.*§	*ml*		
Trial fluid							
Day 1¶	374/397	1500	1000–1500	375/400	1500	1000–2000	0.09
Day 2	288/379	1500	1000–2000	307/380	1500	950–2000	0.50
Day 3	176/330	1000	500–1500	170/326	1000	500–1500	0.78
Open-label trial fluid							
Day 1¶	157/397	1500	1000–2000	177/400	1500	800–2500	0.21
Day 2	114/379	1000	500–1500	133/380	1000	500–2000	0.13
Day 3	54/329	900	500–1000	57/326	1000	500–1250	0.69
Other fluids‖							
Day –1**	356/366	3500	2000–4938	370/385	3000	2000–4868	0.08
Day 1¶	389/394	2235	1325–3197	393/396	1976	1077–3046	0.12
Day 2	373/376	2980	2143–3960	369/371	2905	2094–3780	0.50
Day 3	313/316	3150	2365–3910	315/317	3035	2183–3924	0.33
Blood products††							
Day –1**	90/392	838	480–1435	88/399	600	490–1195	0.69
Day 1¶	109/397	590	300–1100	89/400	600	490–980	0.13
Day 2	115/378	600	350–1100	78/379	526	300–1030	0.001
Day 3	81/327	500	300–980	68/326	598	300–750	0.28
Total‡‡	243/376	1340	566–2700	204/380	1055	600–2755	0.003

* Detailed data on other fluids, blood products, and fluid balances are given in Tables S2 and S3 in the Supplementary Appendix.
† The Wilcoxon signed-rank test was used to compare differences in fluid volume between the starch group and the Ringer's acetate group.
‡ Values are for the patients who received the intervention on the day.
§ The number of patients refers to those who received the specific solution, and the total number refers to those who had data registered. Total numbers that are smaller than the group totals reflect the exclusion of patients who died, were discharged from the ICU, or had missing data.
¶ Day 1 was from the time of randomization to the next start of the 24-hour fluid chart in the ICU; the median duration was 14 hours (interquartile range, 8 to 19).
‖ Other fluids included crystalloids, nutrition, water, fluid with medications, synthetic colloids, and albumin.
** Day –1 refers to the 24 hours before randomization.
†† Blood products included packed red cells, fresh-frozen plasma, and platelet concentrates.
‡‡ The values shown are cumulative data for the full trial period in the ICU, to a maximum of 90 days after randomization.

coagulation was impaired and the use of red cells increased, which may have late adverse effects.[23] A high fraction of HES is taken up and deposited in tissues, where it cannot be metabolized and it acts as a foreign body.[24] Long-term toxic effects of HES deposition have been described in the kidney, liver, and bone marrow.[25-27] Together, all these negative effects of HES may have caused the late deaths observed in our trial and in the VISEP trial.

Colloids are generally considered to be more potent plasma volume expanders than crystalloids. The natural colloid albumin is likely to have a plasma volume–expanding potency that is 40 percent higher than that of saline,[28] but the pharmacokinetics of HES 130/0.42 are different from those of albumin.[24] In this large trial of masked fluid resuscitation with HES 130/0.42 as compared with Ringer's acetate, we did not observe significant

Table 3. Primary and Secondary Outcomes.*

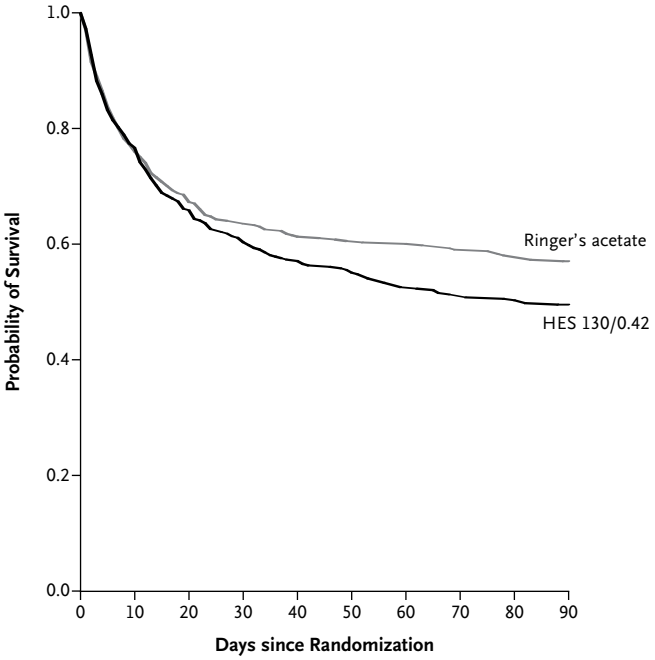
Outcome	HES 130/0.42 (N=398)	Ringer's Acetate (N=400)	Relative Risk (95% CI)	P Value
Primary outcome				
Dead or dependent on dialysis at day 90 — no. (%)	202 (51)	173 (43)	1.17 (1.01–1.36)	0.03
Dead at day 90 — no. (%)	201 (51)	172 (43)	1.17 (1.01–1.36)	0.03
Dependent on dialysis at day 90 — no. (%)	1 (0.25)	1 (0.25)	—	1.00
Secondary outcome measures				
Dead at day 28 — no. (%)	154 (39)	144 (36)	1.08 (0.90–1.28)	0.43
Severe bleeding — no. (%)†	38 (10)	25 (6)	1.52 (0.94–2.48)	0.09
Severe allergic reaction — no. (%)†	1 (0.25)	0	—	0.32
SOFA score at day 5 — median (interquartile range)	6 (2–11)	6 (0–10)	—	0.64
Use of renal-replacement therapy — no. (%)‡	87 (22)	65 (16)	1.35 (1.01–1.80)	0.04
Use of renal-replacement therapy or renal SOFA score ≥3 — no. (%)§	129 (32)	108 (27)	1.20 (0.97–1.48)	0.10
Doubling of plasma creatinine level — no. (%)†	148 (41)	127 (35)	1.18 (0.98–1.43)	0.08
Acidosis — no. (%)†¶	307 (77)	312 (78)	0.99 (0.92–1.06)	0.72
Alive without renal-replacement therapy — mean % of days‖	91	93	—	0.048
Use of mechanical ventilation — no. (%)†	325 (82)	321 (80)	1.02 (0.95–1.09)	0.61
Alive without mechanical ventilation — mean % of days‖	62	65	—	0.28
Alive and out of hospital — mean % of days‖	29	34	—	0.048

* For severe bleeding and severe allergic reaction, data were missing for 1 patient in the Ringer's acetate group. For doubling of the plasma creatinine level, data were missing for 38 patients in the HES 130/0.42 group and 34 patients in the Ringer's acetate group. For alive without mechanical ventilation, data were missing for 1 patient in the Ringer's acetate group. CI denotes confidence interval.
† Outcomes are for patients in the ICU during the 90-day trial period.
‡ Outcomes are for patients with any form of renal-replacement therapy during the 90-day trial period.
§ Outcomes are for patients with any form of renal-replacement therapy during the 90-day trial period or with a renal SOFA score of 3 or higher after the patient had a renal SOFA score of 2 or lower at randomization.
¶ Acidosis was defined as an arterial pH of less than 7.35.
‖ The mean percentage of days was calculated as the number of days without renal-replacement therapy or mechanical ventilation or the number of days out of the hospital divided by the number of days alive in the 90-day follow-up period.

differences in trial-fluid volumes between the study groups, a finding that is in line with the results of a smaller trial that compared HES 130/0.4 (Voluven) with 0.9% saline in patients with sepsis.[29] This finding and the fact that none of the other fluid volumes or balances differed markedly between the groups raises the question of whether there actually is a difference in potency between HES 130/0.42 and crystalloids in patients with severe sepsis.

The strengths of our trial include a low risk of bias, because group assignments were concealed and all trial procedures were blinded. It is reasonable to assume that our results are generalizable, because patients were recruited in university and nonuniversity hospitals with the use of broad inclusion criteria and few exclusion criteria; the majority of screened patients were included. The trial protocol was pragmatic, with routine practice maintained except for fluid resuscitation. In addition, most of the characteristics of the patients were similar to those of ICU patients with sepsis in other trials.[4,30,31] We included more patients who were in shock or mechanically ventilated than have other trials of fluid resuscitation in ICU patients with severe sepsis.[4,31] Outcome rates in our trial were similar to those in previous trials with respect to severe bleeding,[14] use of renal-replacement therapy,[4,31] and mortality.[4,31]

A Time to Death



No. at Risk				
HES 130/0.42	398	240	209	197
Ringer's acetate	400	254	240	228

B Relative Risk of the Primary Outcome

Subgroup	HES 130/0.42	Ringer's Acetate	Relative Risk (95% CI)	P Value for Heterogeneity
	no. of events/no. in subgroup			
Shock at the time of randomization				0.22
Yes	179/336	148/337	1.21 (1.04–1.42)	
No	23/62	25/63	0.93 (0.60–1.46)	
Acute kidney injury at the time of randomization				0.60
Yes	72/142	63/140	1.13 (0.88–1.44)	
No	130/256	110/260	1.20 (1.00–1.45)	
All patients	202/398	173/400	1.17 (1.01–1.36)	

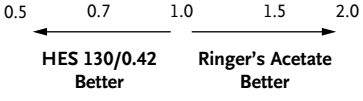


Figure 2. Time to Death and Relative Risk of the Primary Outcome.

Panel A shows the survival curves censored at day 90 for the two intervention groups in the modified intention-to-treat population. Kaplan–Meier analysis showed that the survival time did not differ significantly between the two groups (P=0.07). Panel B shows relative risks with 95% confidence intervals (CIs) for the primary outcome of death or dependence on dialysis at day 90 in the HES 130/0.42 group as compared with the Ringer's acetate group, among all patients and in the two predefined subgroups. Shock at the time of randomization was defined as a mean arterial pressure of less than 70 mm Hg, need for ongoing treatment with vasopressor or inotropic agents, or a plasma lactate level of more than 4.0 mmol per liter in the hour before randomization. Acute kidney injury at the time of randomization was defined as a renal score on the Sepsis-related Organ Failure Assessment (SOFA) of 2 or higher (plasma creatinine level >1.9 mg per deciliter [170 μmol per liter] or urinary output <500 ml) in the 24 hours before randomization. The SOFA score includes subscores ranging from 0 to 4 for each of five organ systems (circulation, lungs, liver, kidneys, and coagulation), with higher scores indicating more severe organ failure.

Our trial has certain limitations. The pragmatic trial design did not include hemodynamic monitoring or cointerventions in the protocol except for recommendations to ask centers to follow international guidelines.[2] Whether this affected the results cannot be assessed. We did not assess all cointerventions during the trial period. Because the trial was large, was blinded, and used stratified randomization, it is less likely that any imbalance in concomitant interventions affected the results. We included patients with acute kidney injury at the time of randomization. Their inclusion is unlikely to have affected the trial results, because acute kidney injury occurred with equal frequency in the two intervention groups and because the effect of HES 130/0.42 did not differ significantly between patients with and those without acute kidney injury at the time of randomization. Seventy-seven patients were given open-label synthetic colloids during the trial period. The use of these agents is unlikely to have affected the results, because the frequency of use was similar in the two intervention groups and because the per-protocol analyses, from which these patients were excluded, supported the primary analysis. Such protocol violations are difficult to prevent in multicenter trials in the ICU, and similar frequencies were observed in the two other large trials of fluid therapy in ICU patients.[4,28] Sixty-nine patients were given trial fluid at doses higher than the maximum daily dose. To limit the potential harm to trial participants from high volumes of HES, we defined the dosage a priori to be lower than that recommended by the manufacturers of HES and used ideal body weight in the dosage calculations. Therefore, only two patients in our trial received HES 130/0.42 at a dose higher than the maximum daily dose recommended by the manufacturers.

In conclusion, patients with severe sepsis who received fluid resuscitation with HES 130/0.42, as compared with those who received Ringer's acetate, had a higher risk of death at 90 days, were more likely to receive renal-replacement therapy, and had fewer days alive without renal-replacement therapy and fewer days alive out of the hospital.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

APPENDIX

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Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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Supplementary Appendix

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Trial fluid composition
6% hydroxyethyl starch (HES) with a molecular weight of 130 kDa and a substitution ratio of 0.42 (6% Tetraspan®, B. Braun Medical AG, Melsungen, Germany). One liter contains HES 130/0.42 60 g, Na+ 140.0 mmol, K^+ 4.0 mmol, Ca^{++} 2.5 mmol, Mg^{++} 1.0 mmol, Cl^- 118.0 mmol, malic acid 5.0 mmol and acetate 24.0 mmol.

Ringer's acetate (Sterofundin ISO®, B. Braun). One liter contains Na+ 145.0 mmol, K^+ 4.0 mmol, Ca^{++} 2.5 mmol, Mg^{++} 1.0 mmol, Cl^- 127.0 mmol, malic acid 5.0 mmol and acetate 24.0 mmol.

Trial definition of fluid resuscitation
Fluid resuscitation was a bolus of intravenous fluid, which was given to increase intravascular volume. The resuscitation fluid should be given in addition to that required to replace ongoing insensible losses, urinary losses etc. or for nutrition.

Trial criteria for severe sepsis
Sepsis was defined as a (1) DEFINED FOCUS OF INFECTION AND (2) at least TWO systemic inflammatory response syndrome (SIRS) criteria.[1]

(1) DEFINED FOCUS OF INFECTION was indicated by either

(i) An organism grown in blood or sterile site

OR

(ii) An abscess or infected tissue (e.g. pneumonia, peritonitis, urinary tract, vascular line infection, soft tissue, etc).

(2) The 4 SIRS criteria were:

1. CORE TEMPERATURE > 38°C or < 36°C. (Core temperature was rectal, urinary bladder, central line, or tympanic). If oral, inguinal or axillary temperatures were used, 0.5°C was added to the measured value. Hypothermia < 36°C was confirmed by core temperature only. We used the most deranged value recorded in the 24 hours before randomization.
2. HEART RATE > 90 beats/minute. If the patient had atrial arrhythmia, the ventricular rate was recorded. If the patients had known medical condition or were receiving treatment that would prevent tachycardia (for example, heart block or beta blockers), they had to meet two of the remaining three SIRS criteria. We used the most deranged value recorded in the 24 hours before randomization.
3. RESPIRATORY RATE > 20 breaths/minute, $PaCO_2$ < 32 mmHg (4.3 kPa) or mechanical ventilation for an acute process. We used the most deranged respiratory rate or $PaCO_2$ recorded in the 24 hours before randomization.
4. WHITE BLOOD CELL COUNT of >12 x 10^9/liter or < 4 x 10^9/liter or > 10% immature neutrophils (band forms). We used the most deranged value recorded in the 24 hours before randomization.

Severe sepsis was defined as SEPSIS plus at least ONE ORGAN FAILURE, except when that organ failure was already present 48 hours before the onset of sepsis.

ORGAN FAILURE was defined as a Sepsis-related Organ Failure Assessment (SOFA) score > 2 for the organ in question (Table S9).[2]

Calculation of the maximum daily dose of trial fluid
The following was calculated electronically for each individual patient in the web-based screening form (Expertmaker, Malmö, Sweden) to reduce the risk of giving too high doses of trial fluid:

- The maximum daily dose of trial fluid was based on estimated ideal body weight (men: estimated height in cm – 100; women: estimated height in cm – 105).
- The calculated maximum daily dose of trial fluid (ideal body weight in kg x 33 ml/kg) was reduced to the nearest 500 ml.
- On the 1st day of the trial, any volume of synthetic colloids given in the 24 hours prior to randomization was subtracted from the calculated maximum daily dose of trial fluid allowed.

Protocol violations
Sixty-nine patients (9%) received trial fluid above the protocolized daily maximum dose (median volume 500 (interquartile range 500-1000) ml), 28 in the HES 130/0.42 group and 41 in the Ringer's acetate group. This occurred mainly on the first trial day (n=45). Only two patients in the HES 130/0.42 group received more than the recommended daily dose by the manufacturers of 50 ml/kg and this occurred on single days only.

Seventy-seven patients received open-label synthetic colloids (67 HES 130/0.42 and 10 dextran 70) in the ICU in the 90-day trial period, 39 in the HES 130/0.42 group and 38 in the Ringer's acetate group.

In 28 cases consent was either not granted or withdrawn by the next of kin or the patient, 17 in the HES 130/0.42 group and 11 in the Ringer's acetate group. This occurred 35 (14-72) hours after randomization during which the patients received 1813 (1000-2500) ml of trial fluid. Continued data registration and use of data was allowed in all these cases.

Trial populations
Intention-to-treat population: All randomized patients. This population was not analyzed in the 6S-trial.

Modified intention-to-treat population: All randomized patients except those who

- Withdrew consent for the use of data

OR

- Were not eligible for randomization according to the inclusion/exclusion criteria AND never had the intervention (masked trial fluid)

Per-protocol populations
Two per-protocol analyses were planned to allow the first analysis to be done before the unblinding of the data. The second per-protocol analysis was done after unblinding the data. In contrast to the first analysis the patients in the HES 130/0.42 group who had received open-label synthetic colloid after randomization were included in the second per-protocol analysis.

Per-protocol population no. 1:
All randomized patients except patients having one or more major protocol violations defined as

- Patients who were not eligible for randomization according to the inclusion/exclusion criteria.

OR

- Patients who never had the intervention (masked trial fluid).

OR

- Patients who accidentally received wrong intervention (intervention error).

OR

- Patients who received any synthetic colloid after randomization.

OR

- Patients who were withdrawn from the protocol because the proxy, the relatives, the general practitioner or the patient himself withdrew consent.

Per-protocol population no. 2:
All randomized patients except patients having one or more major protocol violations defined as
- Patients who were not eligible for randomization according to the inclusion/exclusion criteria.

OR
- Patients who never had the intervention (masked trial fluid).

OR
- Patients who accidentally received wrong intervention (intervention error).

OR
- Patients in the Ringer's acetate arm, who received any synthetic colloid after randomization.

OR
- Patients who were withdrawn from the protocol because the proxy, the relatives, the general practitioner or the patient himself withdrew consent.

Per-protocol analyses
Results of per-protocol analysis no. 1
The per-protocol no. 1 analysis of the primary outcome showed that 673 patients (335 from the HES 130/0.42 group and 338 from the Ringer's acetate group) could be included in this analysis. The primary outcome occurred in 165 (49%) of the patients in the HES 130/0.42 group and in 146 (43%) in the Ringer's acetate group exhibiting an intervention effect of an absolute risk difference of 6% or a relative risk of 1.14 (95% confidence limits: 0.97-1.34, P=0.12)

Results of per-protocol analysis no. 2
The per-protocol no. 2 analysis of the primary outcome showed that 705 patients (367 from the HES 130/0.42 group and 338 from the Ringer's acetate group) could be included in this analysis. The primary outcome occurred in 184 (50%) of the patients in the HES 130/0.42 group and in 146 (43%) in the Ringer's acetate group exhibiting an intervention effect of an absolute risk difference of 7% or a relative risk of 1.16 (95% confidence limits: 0.97-1.37, P=0.07)

Handling of missing data

Logical imputations performed for baseline variables

SAPS II in the 24 hours prior to randomization
The score is based on 17 components each measured in the first 24 hours in the ICU. In the baseline form, we registered values measured before randomization only. Randomization immediately after ICU-admittance therefore resulted in missing values. However, day 1 values measured shortly afterwards may reflect the patient's condition.

Since day 1 ran from randomization until the start of the next "fluid day" of the ward, day 1 had a short duration in some patients. In these situations there were missing data both at baseline and on day 1. However, data from day 2 may reflect the patient's condition in these situations.

Missing PaO_2/FiO_2-ratio: If the patient was randomized within 24 hours after ICU-admittance, values from day 1 were used for SAPS-scoring.

Missing diuresis: If the patient was randomized within 24 hours after ICU-admittance AND creatinine < 100 µmol/liter (1.2 mg/deciliter) AND diuresis on day 1 > 1000 ml, the patient's kidney function was considered normal and the patient was given zero points.

Missing leucocytes: If the leucocytes were reported in the normal range in the screening form zero points were given.

Missing bilirubin: The value from day 1 was used. If this value was also missing zero points were given, if the doctor had reported normal bilirubin in the screening form.

The above imputations reduced the number of incomplete SAPS II values from 296 to 213.

For the remaining 213 patients 'best' and 'worst' scores were calculated covering all possible true scenarios. Setting missing SAPS-components to zero points made the 'best' possible score.

Patients were given the highest obtainable points for the calculation of the 'worst' possible score. However, for Glasgow Coma Scale (GCS) and blood pressure the imputation depended on other data as well:

If GCS score was < 13 in the screening form, 26 points were imputed, otherwise only 5 points were imputed.

If the lowest mean arterial pressure at baseline was >70 mmHg, then the systolic blood pressure must also have been > 70 mmHg and 5 points were imputed instead of 13 points.

SOFA score in the 24 hours prior to randomization
This score does not depend on when the patient was admitted to the ICU.

Missing renal component: No missing values.

Missing platelet count: Values from day 1 were used; otherwise from day 2.

Missing plasma bilirubin: Values from day 1 were used; otherwise from day 2. If still missing, the patient got zero points if the doctor had reported normal bilirubin in the screening form.

Missing PaO_2/FiO_2-ratio: Values from day 1 were used.

Missing cardiovascular component: One missing value. According to the screening form the patient had normal blood pressure and did not receive any vasopressors or inotropes. This patient was given 0 points.

The above imputations reduced the number of incomplete SOFA scores from 121 to 2.

Missing outcome data
For the primary outcome measure and most of the secondary outcomes we had full data sets on all 798 patients.

There were missing data for the following secondary outcome measures:

Doubling of plasma creatinine because 62 patients had no pre-admission plasma creatinine (33 and 29 patients in the HES 130/0.42 and Ringer's acetate groups, respectively), 9 patients died early and had no creatinine measured after randomization and one patient had source data missing for five ICU days (Ringer's acetate group). We did a complete case analysis of this outcome (726 patients).

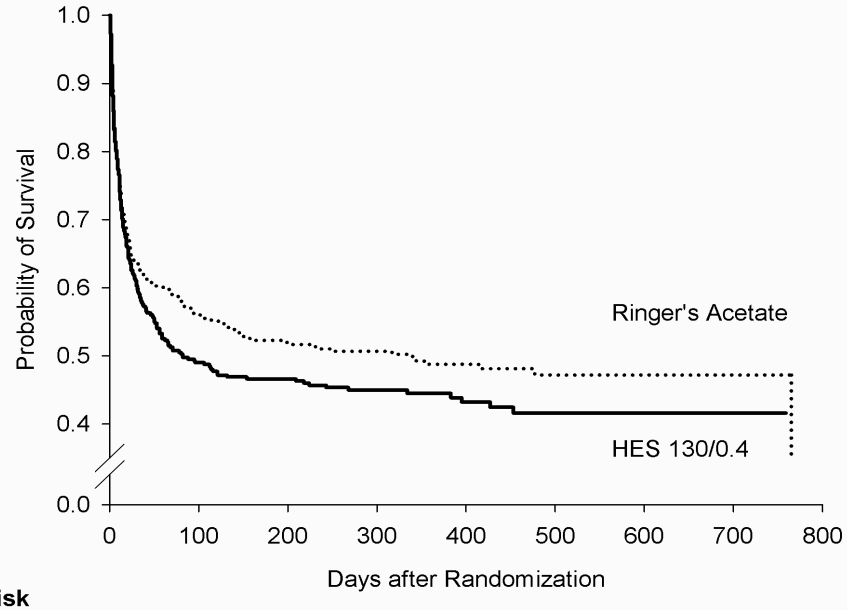
Severe bleeding because one patient had source data missing for five ICU days (Ringer's acetate group). We did a complete case analysis of this outcome (797 patients).

Severe allergic reaction because one patient had source data missing for five ICU days (Ringer's acetate group). We did a complete case analysis of this outcome (797 patients).

Days alive without mechanical ventilation because one patient had source data missing for five ICU days (Ringer's acetate group). We did a complete case analysis of this outcome (797 patients).

Abstract written before breaking the randomization code
BACKGROUND
Hydroxyethyl starch (HES) 130/0.4 is widely used for fluid resuscitation in intensive care units (ICU), but largely unstudied in patients with severe sepsis.
METHODS
In this multicenter, parallel group, blinded trial, we randomly assigned patients with severe sepsis to fluid resuscitation in the ICU using either 6% HES 130/0.4 or Ringer's acetate up to 33 milliliter/kg/day. The primary outcome measure was either death or end-stage kidney failure 90 days after randomization and secondary outcomes included acute kidney failure, need of dialysis and severe bleeding.
RESULTS
Of the 804 randomized patients, 798 were included in the modified intention-to-treat population. The two intervention groups had comparable baseline characteristics. At 90 days after randomization, 202 of the 398 patients (51%) assigned to 0 fulfilled the primary outcome of death or end-stage kidney failure compared with 173 of the 400 patients (43%) assigned to 1, relative risk 1.17 (95% confidence interval 1.01 – 1.36; P=0.034). Also 90-day mortality and need of dialysis was higher and days alive without dialysis and days alive and out of hospital was lower in the patients in the 0 group compared with those in the 1 group. The results were confirmed in multivariate analyses adjusting for known risk factor at baseline and in per protocol analyses.
CONCLUSIONS
Patients with severe sepsis who were fluid resuscitated with 0 had higher 90-day mortality and need of dialysis and fewer days alive without dialysis and out of hospital compared with those receiving 1.

Figure S1. Time to Death Analysis
Shown are the survival curves censored at latest follow-up on February 16th 2012 for the two intervention groups in the modified intention-to-treat population. Kaplan Meier analysis showed that the survival time did not differ significantly between the groups (P=0.14).



No. at Risk								
HES 130/0.4	398	193	152	104	67	34	12	5
Ringer's Acetate	400	224	169	125	81	46	19	9

Table S1. More Baseline Characteristics

	HES 130/0.42 (N=398)	Ringer's Acetate (N=400)
Actual body weight – kg	77 (65-89)	76 (65-86)
Diabetes mellitus – no. (%)	52 (13)	57 (14)
Arterial hypertension – no. (%)	156 (39)	156 (39)
Previous admission for – no. (%)		
Heart failure or myocardial infarction	49 (12)	62 (16)
Stroke	31 (8)	42 (11)
Asthma or COPD	60 (15)	58 (15)
Pre-admission plasma creatinine > 100 µmol/liter (1.2 mg/deciliter) – no. (%)	57 (14)	64 (16)
Hematological malignancy – no. (%)	36 (9)	36 (9)
Positive culture from blood or a sterile site – no. (%)	81 (20)	82 (21)
Time from ICU admission to randomization – hours	3.7 (1.3-12.9)	4.0 (1.4-12.6)
Organ failures *		
Cerebral failure †	135 (34)	121 (30)
Respiratory failure	289 (73)	293 (73)
Circulatory failure	259 (65)	252 (63)
Hepatic failure	47 (12)	44 (11)
Kidney injury	142 (36)	140 (35)
Coagulation failure	81 (20)	74 (19)
Use of potential nephrotoxic agents §	118 (30)	120 (30)
Use of synthetic colloids – no. (%) ¶	169 (42)	168 (42)
Volume of synthetic colloids – ml ¶	700 (500-1000)	500 (500-1000)

Values with ranges are medians (interquartile ranges).
COPD denotes chronic obstructive pulmonary disease, HES hydroxyethyl starch, ICU intensive care unit.
*Defined as Sepsis-related Organ Failure Assessment score of 2 or above in the given organ system at randomization (Table S9).[2] Most patients had two or more failing organ systems.
† Glasgow Coma Scale (GCS) score < 13 without a structural cause. If the patient was sedated, the GCS score estimated before sedation was used.
§ Any of the following agents given during hospital admission but prior to randomization: IV gentamicin, IV vancomycin, IV amphotericin B, IV polymyxins, IV dye contrast, ciclosporin A, non-steroid anti-inflammatory drugs, ganciclovir, tacrolimus, ifosfamid, atripla, or candesartancilexetil.
¶ Hydroxyethyl starch, gelatin, or dextran given in the 24 hours prior to randomization. Volumes given are medians (interquartile ranges) for those receiving colloids.

Table S2. Details on Fluid Therapy, Blood Products, and Nutrition

Variable	HES 130/0.42 (N=398)		Ringer's Acetate (N=400)		P Value
	No. receiving / No. at risk †	Value	No. receiving / No. at risk †	Value	
Albumin (ml)					
Day -1 ¶	36/391	500 (250-625)	38/397	275 (250-750)	0.87
Day 1 ‡	15/397	250 (200-500)	14/399	250 (200-300)	0.85
Day 2	15/379	300 (200-500)	12/380	325 (150-700)	0.56
Day 3	15/328	200 (100-500)	14/326	200 (100-300)	0.86
Total §	80/379	500 (225-1200)	65/381	400 (250-1000)	0.14
Crystalloids (ml)					
Day -1 ¶	350/373	2500 (1400-4000)	349/386	2400 (1400-4000)	0.20
Day 1 ‡	235/397	1000 (525-2000)	223/399	1000 (500-2000)	0.22
Day 2	162/378	740 (250-1397)	136/379	1000 (500-1510)	0.15
Day 3	125/322	800 (200-1060)	101/323	850 (400-1500)	0.10
Total §	310/363	2500 (1000-6000)	290/358	2300 (1000-4970)	0.05
Packed red blood cells (ml)					
Day -1 ¶	71/392	550 (300-1045)	65/399	500 (300-900)	0.50
Day 1 ‡	84/397	490 (279-600)	59/400	490 (275-840)	0.03
Day 2	82/378	490 (250-600)	54/379	300 (245-510)	0.005
Day 3	53/328	300 (245-500)	43/326	490 (250-600)	0.35
Total §	220/377	900 (490-1715)	173/380	900 (551-1715)	0.005
Fresh frozen plasma (ml)					
Day -1 ¶	42/392	600 (540-1113)	38/399	600 (540-1080)	0.57
Day 1 ‡	44/397	600 (540-800)	41/400	600 (540-1080)	0.76
Day 2	47/378	700 (540-1080)	30/380	560 (540-813)	0.03
Day 3	28/328	540 (526-950)	18/326	585 (528-1080)	0.14
Total §	113/377	1080 (540-1815)	96/382	950 (540-2165)	0.14
Platelets (ml)					
Day -1 ¶	25/392	600 (350-700)	22/399	480 (350-1050)	0.62
Day 1 ‡	22/397	350 (300-600)	21/400	350 (350-700)	0.88
Day 2	31/378	400 (350-700)	22/380	700 (350-710)	0.21
Day 3	24/327	675 (350-735)	27/326	600 (350-700)	0.69
Total §	71/376	700 (350-2100)	53/382	1000 (650-3500)	0.09
Nutrition (ml) **					
Day 1 ‡	242/396	638 (315-1102)	248/399	632 (300-1089)	0.77
Day 2	321/378	1250 (870-1622)	321/378	1192 (775-1600)	0.46

Day 3	296/321	1490 (1000-1855)	294/321	1478 (947-1785)	0.34
Total §	308/350	8609 (3125-19256)	299/346	7666 (2500-19194)	0.34

Values are medians (interquartile ranges) of those patients who did receive the intervention on that day(s). HES denotes hydroxyethyl starch.
† No. receiving is those patients who did receive the specific solution on the given day(s). No. at risk is those patients who had data registered. Where the no. is below the no. allocated to the group this is due to death, ICU discharge or missing source data.
¶ In the 24 hours prior to randomization.
‡ The first day was from the time of randomization to the next start of the specific ICU's 24-hour fluid chart and lasted median 14 (8-19) hours.
§ Cumulative data for the full trial period in the ICU to a maximum of 90 days after randomization.
** Added volumes of enteral and parenteral nutrition including any glucose solution > 9% and any protein or lipid solutions.

Table S3. Urinary Outputs and Fluid Balances

Variable	HES 130/0.42 (N=398)		Ringer's Acetate (N=400)		P Value
	No. with data / No. at risk †	Value	No. with data / No. at risk †	Value	
Urine output (ml)					
Day 1 ‡	394/398	1938 (1000-2860)	396/400	1800 (920-2820)	0.31
Day 2	377/380	2150 (1195-2950)	374/382	2348 (1395-3300)	0.03
Day 3	321/331	2400 (1430-3300)	321/327	2550 (1595-3500)	0.17
Total §	333/398	14890 (5340-32480)	331/400	13700 (5720-32550)	0.69
Fluid balance (ml)					
Day 1 ‡	387/398	2206 (941-3895)	391/400	2200 (919-3798)	0.92
Day 2	372/380	1828 (625-3355)	367/382	1656 (510-3043)	0.13
Day 3	310/331	975 (1-2145)	314/327	765 (-90-1964)	0.31
Total §	288/398	5452 (1876-10518)	291/400	4616 (1271-9530)	0.17

Values are medians (interquartile ranges) of those patients who had data registered on that day(s).
HES denotes hydroxyethyl starch.
† No. with data is those patients where data were registered for that day(s). No. at risk is those patients who were in the ICU on that day(s). Where the no. is below the no. allocated to the group this is due to death or ICU discharge.
‡ The first day was from the time of randomization to the next start of the specific ICU's 24-hour fluid chart and lasted median 14 (8-19) hours.
§ Cumulative data for the full trial period in the ICU to a maximum of 90 days after randomization.

Table S4. Circulatory Parameters at Baseline and in the First 24 Hours after Randomization

Variable	HES 130/0.42 (N=398)		Ringer's Acetate (N=400)		P Value
	No. assessed †	Value	No. assessed †	Value	
CVP – mm Hg					
Baseline	110	10 (7-13)	101	10 (8-13)	0.26
0 – 12 hours ‡	151	11 (7-14)	146	10 (7-13)	0.37
12 – 24 hours ‡	129	11 (6-14)	125	10 (6-13)	0.16
$ScvO_2$ – %					
Baseline	175	75 (67-83)	152	73 (65-82)	0.13
0 – 12 hours ‡	181	72 (66-77)	193	73 (65-78)	0.84
12 – 24 hours ‡	131	75 (68-79)	133	73 (67-79)	0.48
Lactate – mmol/liter					
Baseline	385	2.0 (1.3-3.5)	387	2.1 (1.4-3.7)	0.34
0 – 12 hours ‡	390	2.2 (1.4-3.9)	393	2.2 (1.5-3.6)	0.84
12 – 24 hours ‡	337	2.0 (1.3-3.3)	338	2.0 (1.4-2.8)	0.40

*Values are medians (interquartile ranges)
CVP denotes central venous pressure, HES hydroxyethyl starch, $ScvO_2$ central venous oxygen saturation.
† Number of patients where the measurements were documented in source data.
‡ Hours after randomization. Where more measurements were documented within the time period the lowest value of CVP and $ScvO_2$ and the highest value of lactate were registered

Table S5. Use of Potential Nephrotoxic Agents in the ICU after Randomization

	HES 130/0.42 (N=398)	Ringer's Acetate (N=400)
IV gentamicin	14 (4)	25 (6)
IV vancomycin	78 (20)	85 (21)
IV amphotericin B	12 (3)	20 (5)
IV polymyxins	11 (3)	14 (4)
IV dye contrast	73 (18)	66 (17)
Ciclosporin A	2 (1)	5 (1)
NSAIDs	10 (3)	9 (2)
Others †	12 (3)	11 (3)

Values are number of patients (%)
HES denotes hydroxyethyl starch IV denotes intravenous, NSAIDs non-steroid anti-inflammatory drugs.
† Others include tacrolimus, voriconazole, anidulafungin, foscarnet and candesartancilexetil.

Table S6. Results of the Adjusted Analyses

	Best case scenario			Worst case scenario		
Quantity	OR	95% CI	P value	OR	95% CI	P value
Intervention (reference: no HES)	1.53	1.13 – 2.07	0.005	1.35	1.00 – 1.81	0.05
Age/year	1.03	1.02 – 1.05	<0.001	1.03	1.02 – 1.04	<0.0001
Inclusion at a university hospital (reference: not)	0.82	0.60 – 1.11	0.20	0.80	0.59 – 1.09	0.16
Diabetes (reference: not)	0.53	0.34 – 0.83	0.005	0.53	0.34 – 0.83	0.005
Hematological malignancy (reference: not)	1.79	1.02 – 3.13	0.04	1.83	1.05 – 3.19	0.03
Shock (reference: not)	1.14	0.74 – 1.76	0.54	1.20	0.78 – 1.84	0.41
Pre-admission renal dysfunction (reference: not)	1.51	0.99 – 2.31	0.06	1.58	1.04 – 2.42	0.03
Use of nephrotoxic drugs (reference: no drugs)	0.83	0.60 – 1.16	0.28	0.85	0.61 – 1.17	0.32
SOFA score excluding GCS score > 7	1.38	1.00 – 1.90	0.05	1.31	0.94 – 1.81	0.11
SAPS II > 50	1.81	1.31– 2.51	<0.001	1.94	1.140– 2.68	<0.0001

CI denotes confidence intervals, GCS Glasgow Coma Scale, HES hydroxyethyl starch, OR odds ratios, SAPS Simplified Acute Physiology Score, SOFA Sepsis-related Organ Failure Assessment.
Odds ratios and 95% confidence intervals for the intervention with HES forcing adjusting co-variates at baseline into the multivariate analysis of the primary outcome of death and dialysis-dependency 90 days after randomization. There were missing values for SAPS II, so sensitivity analyses were performed using best- and worst case scenarios to test the results of the multiple logistic regression analyses.

Table S7. Results of Post-hoc Analyses of Kidney Injury after Randomization

Mortality data for patients with post-randomization acute kidney injury* and patients treated with renal replacement therapy divided by allocation group

The interpretations of these post-hoc analyses are difficult because of the likely interaction between the HES treatment and AKI and the possible interaction between AKI (oliguria) and trial fluid administration by clinicians.[3]

Both groups

	Total no.	No. of deaths	Mortality			Total no.	No. of deaths	Mortality
RRT	152	92	61%		AKI	237	153	65%
No RRT	646	281	44%		No AKI	560	220	39%
HES 130/0.42 group								
RRT	87	57	66%		AKI	129	85	66%
No RRT	311	144	46%		No AKI	269	116	43%
Ringer's Acetate group								
RRT	65	35	54%		AKI	108	68	63%
No RRT	335	137	41%		No AKI	291	104	36%

AKI denotes acute kidney injury, HES hydroxyethyl starch, RRT renal replacement therapy
*AKI defined as kidney SOFA score > 2 (Table S9)[2] or use of RRT.

Creatinine-based RIFLE Scoring[4]

	HES 130/0.42 (n=398)		**Ringer's Acetate (n=400)**	
	No.	%	No.	%
Normal kidney function	156	43	163	45
Risk	52	14	73	20
Injury	62	17	53	15
Failure	84	23	67	18
Loss	7	2	9	3
ESKD	1	0.3	1	0.3
	362		366	

ESKD denotes end-stage kidney disease, HES hydroxyethyl starch.
There were missing data for 70 patients: One patient had missing source data for 5 days in the ICU, nine patients died early and had no creatinine measured after randomization, and 62 patients did not have a pre-admission creatinine. However, two of these patients were treated with RRT > 28 days and thereby had Loss.

Creatinine-based RIFLE Scoring - substitution using the MDRD-equation[4]				
	HES 130/0.42 (n=398)		Ringer's Acetate (n=400)	
	No.	%	No.	%
Normal kidney function	167	42	171	43
Risk	60	15	80	20
Injury	69	18	59	15
Failure	90	23	74	19
Loss	7	2	9	2
ESKD	1	0.3	1	0.3
	394		394	

ESKD denotes end-stage kidney disease, HES hydroxyethyl starch, MDRD modification of diet in renal disease.
There were missing data for 10 patients: One patient had missing source data for 5 days in the ICU and nine patients died early and had no creatinine measured after randomization.

Doubling in p-creatinine OR Renal replacement therapy	HES 130/0.42 (N=398)		Ringer's Acetate (N=400)	
	No.	%	No.	%
Yes	175	44	147	37
No	223	56	253	63
Total	398		400	
				P Value 0.04

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Table S8. Results of Post-hoc Analyses of Bleeding after Randomization

Any Bleeding	**HES 130/0.42 (N=398)**		**Ringer's Acetate (N=400)**	
	No.	%	No.	%
Yes	93	23	60	15
No	305	77	339	85
Total	398		399	
				P Value 0.003

There were missing data for one patient in the Ringer's group, who had missing source data for 5 days in the ICU.

PAPER II

Bleeding and Risk of Death with Hydroxyethyl Starch in Severe Sepsis

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Abstract

Purpose: We aimed to characterize the degree and clinical importance of bleeding with hydroxyethyl starch (HES).

Methods: In post-hoc analyses, we examined the associations between fluid assignment, hemostatic variables, bleeding events and death among 798 patients with severe sepsis randomized to fluid resuscitation with HES 130/0.42 versus Ringer's acetate. We used Cox regression analysis adjusted for fluid assignment and baseline characteristics.

Results: Overall, 93 (23%) patients assigned to HES versus 60 (15%) patients assigned to Ringer's acetate bleed in the ICU (relative risk (RR) 1.55; 95% CI 1.16-2.08; p=0.003). Of these, 38 and 25 patients, respectively, had severe bleeding (intracranial or concomitant transfusion with three units of red blood cells). Most patients bleed in the first days after randomization when most trial fluid was given. In this period, the international normalized ratio was higher and hemoglobin levels lower in the HES group. The hazards ratios for occurrence of any bleeding and severe bleeding with HES versus Ringer's acetate were 1.70 (95% CI, 1.23 to 2.36; P=0.001) and 1.55 (95% CI, 0.93 to 2.56; P=0.09), respectively. The adjusted hazard ratios for death among patients with any bleeding and severe bleeding compared to those without bleeding were 1.36 (95% CI, 1.04 to 1.79; P=0.03) and 1.74 (95% CI, 1.20 to 2.53; P=0.004), respectively.

Conclusions: In patient with severe sepsis, treatment with HES increased the risk of bleeding which was associated with increased risk of death. HES induced coagulopathy and bleeding may negatively affect outcome in patients with severe sepsis.

6S Trial ClinicalTrials.gov number, NCT00962156

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Introduction

Hydroxyethyl starch (HES) was the most commonly used colloid in a recent world-wide point prevalence study of resuscitation in intensive care units (ICU) [1]. Systematic reviews report that HES affects hemostasis more than other fluids, but most trials in these reviews assessed the former high molecular weight HES in surgical patients [2, 3]. Thus, the degree of hemostatic impairment in patients with sepsis with the currently used HES with low molecular weight (130.000 Da) and substitution ratio of approximately 0.4 (range 0.38 to 0.45) is unknown, and whether such impairment affects patient important outcomes is yet to be elucidated.

We previously reported that patients with severe sepsis assigned to fluid resuscitation with 6% HES 130/0.42 versus Ringer's acetate had increased risk of death,

bleeding and of being transfused with red blood cells [4]. However, the two latter were not originally protocolized outcomes.

To better understand these results, we analyzed the trial database to further explore the relationships between type of trial fluid, hemostatic variables, bleeding, and mortality.

Methods

Study oversight

The present study is a post-hoc analysis of the Scandinavian Starch for Severe Sepsis / Septic Shock (6S) trial database. The trial protocol, the statistical analysis plan and primary trial results were previously published [4, 5]. The trial was approved by the medicine agencies, ethical committees and data protection agencies in Denmark, Norway, Finland and Iceland prior to randomization of the first patient. Informed consent was obtained prior to randomization from all participants or their legal substitutes according to national legislation. Independently of funding agencies, the authors designed the study, analyzed the data, wrote the manuscript, and made the decision to submit the manuscript for publication. The authors vouch for the accuracy and completeness of the reported data.

Study participants

This randomized trial with concealed allocation and blinding recruited 798 patients with severe sepsis in ICUs in 26 hospitals in Denmark, Norway, Finland and Iceland in 2010 and 2011. Eligible patients fulfilled the criteria for severe sepsis and needed fluid resuscitation as judged by the treating clinician. We excluded patients undergoing renal replacement therapy or having intracranial bleeding. A detailed description of in- and exclusion criteria can be found in the main publication of the 6S trial [4]. Patients were randomly assigned to fluid resuscitation with either 6% HES 130/0.42 in Ringer's acetate (Tetraspan 6%, B Braun, Melsungen, Germany) or Ringer's acetate (Sterofundin ISO, B Braun) to a maximum daily dose of 33 ml per kg ideal body weight per day followed by Ringer's acetate if needed. Randomization was stratified according to the presence or absence of shock, the presence or absence of active hematologic cancer and admission to a university or non-university hospital. The trial design was pragmatic so fluid resuscitation was at the discretion of the treating clinicians, and no other part of the treatment was protocolized. The intervention period lasted until discharge from the ICU to a maximum of 90 days.

At baseline we collected data on demographics and clinical characteristics. Daily recordings during the entire admission to the ICU included bleeding events (see Supplementary Appendix for case record form) and transfusions of red blood cells, platelets and fresh frozen plasma. The lowest hemoglobin levels, the highest international normalized ratio (INR), and the lowest platelet count were registered in the first 5 days. Time of death was registered for all patients to a maximum follow-up of 90 days.

Severe bleeding was defined as intracranial bleeding or bleeding with concomitant transfusion of three units of red blood cells.

Statistical analyses

We examined the influence of the trial fluid on time to any bleeding and on time to severe bleeding using proportional hazards methods (Cox regression analysis), where patients were censored at discharge from the ICU, death or 90 days whichever came first. If patients were discharged from the

ICU to a general ward and readmitted to the ICU, the time between such two ICU admissions was included as observation time with no events. We calculated unadjusted hazard ratios and hazard ratios adjusted for the stratification variables [6], and the following other baseline characteristics: surgery prior to ICU admission or not, HES given in the 24 hours prior to randomization or not, simplified acute physiology score (SAPS) II [7], sepsis-related organ failure assessment (SOFA) score [8], platelet count and INR.

We then assessed the relationship between any bleeding or severe bleeding and mortality within 90 days with Cox regression analysis where occurrence of bleeding was included as a time-dependent covariate. To further examine whether bleedings contributed to the excess mortality observed with HES, we performed a Cox regression analysis of time to death

Table 1 Blood Loss and Relative Risk of Bleeding and Severe Bleeding in the ICU According to Trial Fluid Assignment and Anatomical Site

Anatomical Site	HES 130/0.42 (n=398)	Ringer's Acetate (n=400)	Relative Risk (95%-CI)	P Value
Intracranial bleeding – no. (%)	2 (1)	5 (1)	0.40 (0.08-2.05)	0.45
Upper GI bleeding – no. (%)				
Any	34 (9)	18 (5)	1.89 (1.09-3.30)	0.02
Severe	9 (2)	7 (2)	1.29 (0.48-3.43)	0.61
Lower GI bleeding – no. (%)				
Any	15 (4)	13 (3)	1.16 (0.56-2.40)	0.69
Severe	10 (3)	5 (1)	2.01 (0.69-5.81)	0.19
Urinary tract bleeding – no. (%)				
Any	5 (1)	2 (1)	2.51 (0.49-12.8)	0.29
Severe	3 (1)	1 (0.3)	3.01 (0.31-28.8)	0.37
Lower airway bleeding – no. (%)				
Any	11 (3)	12 (3)	0.92 (0.41-2.06)	0.84
Severe	1 (0.3)	4 (1)	0.25 (0.03-2.23)	0.37
Bleeding from wounds – no. (%)				
Any	27 (7)	18 (5)	1.50 (0.84-2.69)	0.16
Severe	11 (3)	10 (3)	1.10 (0.47-2.57)	0.82
Bleeding during surgery – no. (%)				
Any	33 (8)	26 (7)	1.27 (0.78-2.09)	0.34
Severe	18 (5)	11 (3)	1.64 (0.78-3.43)	0.18
Total – no. (%)				
Any	93 (23)	60 (15)	1.55 (1.16-2.08)	0.003
Severe	38 (10)	25 (6)	1.52 (0.94-2.48)	0.09
Blood loss from any bleeding				
Patients with data – no. (%)	53 (57)	34 (57)		
Volume – ml				0.31
Median	600	800		
Interquartile range	190-2000	400-2500		
Blood loss during surgery				
Patients with data – no. (%)	33 (100)	23 (88)		
Volume – ml				0.78
Median	1650	1000		
Interquartile range	250-2500	200-2600		

The number of patients with any bleeding includes those with severe bleeding. HES denotes hydroxyethyl starch, ICU intensive care unit and GI gastro-intestinal.

according to trial fluid assignment with and without censoring of patients with bleedings.

Risk factors for any bleeding and severe bleeding were identified with the use of univariate and multivariate logistic regression analysis. Covariates were included in the multivariate model if the P value was less than 0.10 in the univariate analysis.

The time courses of the lowest hemoglobin level, highest INR and lowest platelets count during the first 5 days after randomization were analyzed as the difference between the intervention groups in area under the curve and using a mixed model taking into consideration repeated measurements made in the same patient.

In all analyses we used multiple imputation of missing variables according to the recommendations of the Patient-Centered Outcomes Research Institute and Shafer [9, 10].

Author and statistician PW performed the analyses in SPSS 18 and SAS 9.2. A two-sided P value of less than 0.05 was considered to indicate statistical significance.

Results

Study participants

We enrolled 798 patients with severe sepsis in the ICU (Table S1 in the Supplementary Appendix). The observation period for bleeding (time from randomization to discharge from the ICU) was 6 (interquartile range (IQR), 3 to 15) days in the HES group and 7 (IQR, 3 to 15) days in the Ringer's acetate group.

Intervention

Overall, 779 patients (98%) received trial fluid. The median volume of trial fluid on days 1, 2 and 3 were 1500 ml, 1500 ml and 1000 ml, respectively, in both groups. The median cumulative volume during the entire ICU-admission was 3000 ml (IQR, 1507 to 5100) in the HES group and 3000 (IQR, 2000 to 5750) in the Ringer's group (P=0.20) corresponding to 44 and 47 ml per kg ideal body weight, respectively (P=0.18).

Time Course of INR, Hemoglobin Level and Platelet Count

Patients assigned to HES had statistically significant lower hemoglobin and higher INR values than those assigned to Ringer's acetate. The differences were present during the first days after randomization and seemed to diminish towards day 5. The platelet counts were not affected with statistical significance by the type of trial fluid (Fig. 1 and figure in the Supplementary Appendix).

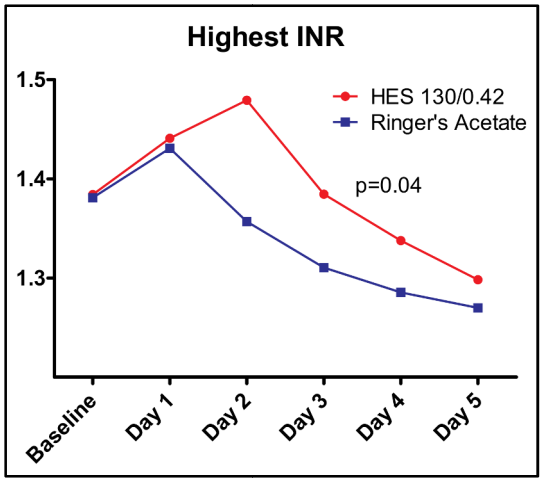

Fig 1 Time course of highest International Normalized Ratio (INR) from baseline till five days after Randomization. The curves show median values for each treatment group. The P value is for difference in area under the curve. We also examined the time courses using a mixed model adjusted for stratification variables and baseline values. We assumed unstructured covariance and found that type of trial fluid associated with INR (P<0.02)

Sites, Rates and Timing of Bleeding

Overall, 93 (23%) patients assigned to HES versus 60 (15%) patients assigned to Ringer's acetate bleed in the ICU (P=0.003). Of these, 38 and 25 patients, respectively, had severe

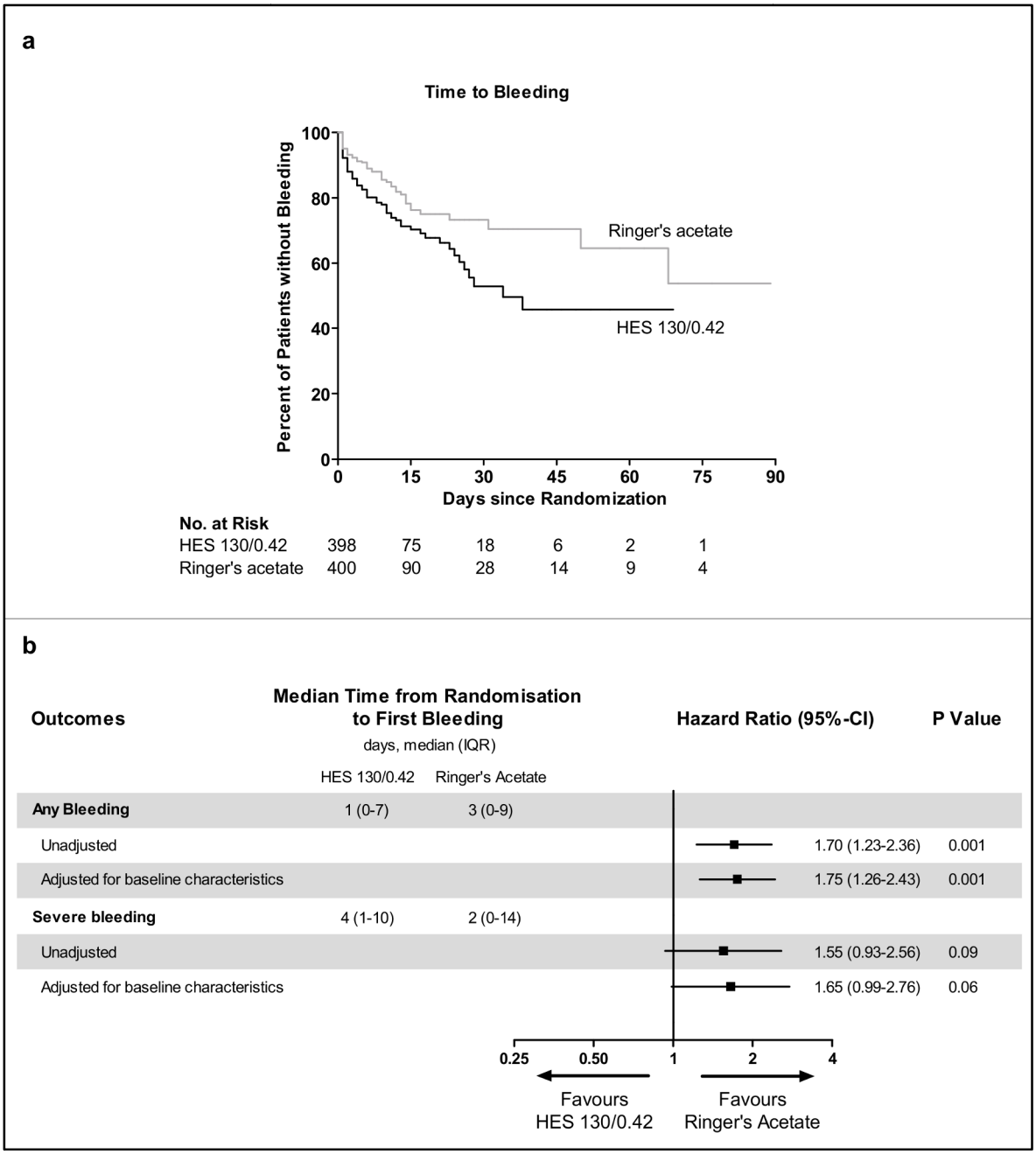

Fig 2 Time to Bleeding and Hazard Ratio for Bleeding and Severe Bleeding According to Trial Fluid Assignment. Panel a shows Kaplan-Meier curves of time to bleeding censored at death, discharge from the intensive care unit or at 90 days whichever came first for the two intervention groups. Kaplan-Meier analysis showed that the time to bleeding differed significantly between the groups (P=0.001). Panel b shows the hazard ratios with 95% confidence intervals for bleeding and severe bleeding according to trial fluid assignment.

bleeding (P=0.09). In both groups most patients had their first bleeding episode within the first days after randomization (Fig. 2), but the longer the patient stayed in the ICU the higher the risk of bleeding (Table S2 in the Supplementary Appendix). Most frequently the patients bleed during surgery, from wounds or from the upper gastrointestinal tract, but the increased risk of bleeding in the HES group seemed independent of bleeding site (Table 1). Once a patient bleed, the duration of the bleeding (median 1 day) and the corresponding estimated blood loss were comparable between the groups (Table 1).

Table 2 Results of Uni- and Multivariate Analysis for Potential Risk Factors at Baseline for Subsequent Bleeding or Severe Bleeding

	Any Bleeding				Severe Bleeding			
Variable	**Odds ratio (95% CI)**	**P Value in univariate analysis**	**Odds ratio (95%-CI)**	**P Value in multivariate analysis**	**Odds ratio (95%-CI)**	**P Value in univariate analysis**	**Odds ratio (95%-CI)**	**P Value in multivariate analysis**
HES 130/0.42 vs. Ringer's acetate	1.73 (1.21 to 2.48)	0.003	1.80 (1.25-2.60)	0.002	1.58 (0.81 to 2.68)	0.09	1.64 (0.96-2.80)	0.07
Admitted to a university hospital	1.79 (1.25 to 2.55)	0.002	1.49 (1.03-2.17)	0.04	2.15 (1.26 to 3.69)	0.005	1.76 (1.01-3.06)	0.047
Surgery prior to ICU admission	1.85 (1.29 to 2.64)	0.001	1.93 (1.32-2.81)	0.001	2.21 (1.32 to 3.72)	0.003	2.28 (1.32-3.92)	0.003
Septic shock	1.49 (0.88 to 2.54)	0.14	-	-	1.30 (0.60 to 2.80)	0.50	-	-
Active Hematologic Cancer	1.58 (0.91 to 2.76)	0.11	-	-	1.78 (0.85 to 3.77)	0.13	-	-
Treatment with HES in the 24 hours prior to randomization	0.91 (0.64 to 1.32)	0.63	-	-	1.11 (0.66 to 1.87)	0.70	-	-
Square root of SAPS II[b]	1.15 (0.97 to 1.37)	0.11	-	-	1.19 (0.92 to 1.53)	0.22	-	-
Square root of SOFA score[c]	1.52 (1.09 to 2.10)	0.01	1.36 (0.93-1.98)	0.11	1.43 (0.89 to 2.29)	0.14	-	-
Transformed INR[d]	1.36 (0.85 to 2.15)	0.20	-	-	1.57 (0.85 to 2.92)	0.16	-	-
Logarithm of platelet count[e]	0.83 (0.69 to 0.98)	0.04	0.88 (0.72-1.09)	0.24	0.80 (0.63 to 1.01)	0.07	0.77 (0.60-1.00)	0.048

The factors included in the multivariate analyses were those with P values less than 0.10 in the univariate analysis. The values for the Simplified Acute Physiology Score (SAPS) II, Sepsis-related Organ Failure Assessment (SOFA) score, International normalized ratio (INR) and platelet count pertain to the 24 hours prior to randomization. HES denotes hydroxyethyl starch and ICU intensive care unit.

[b] SAPS II values were transformed by square root to normalize variance. The odds ratio is per 1-unit increase in the transformed variable. Multiple imputation was used in 105 patients in the starch group and 107 patients in the Ringer's acetate group with missing SAPS II.

[c] The SOFA scoring was modified because cerebral failure was not assessed. SOFA scores were transformed by square root to normalize variance. The odds ratio is per 1-unit increase in the transformed variable.

[d] INR was transformed as the lowest value of either the natural logarithm of INR or 2.12. The odds ratio is per 1-unit increase in the transformed variable. Multiple imputation was used in 27 patients in the starch group and 26 patients in the Ringer's acetate group with missing INR.

[e] Platelet counts were transformed by natural logarithm to normalize variance. The odds ratio is per 1-unit increase in the transformed variable.

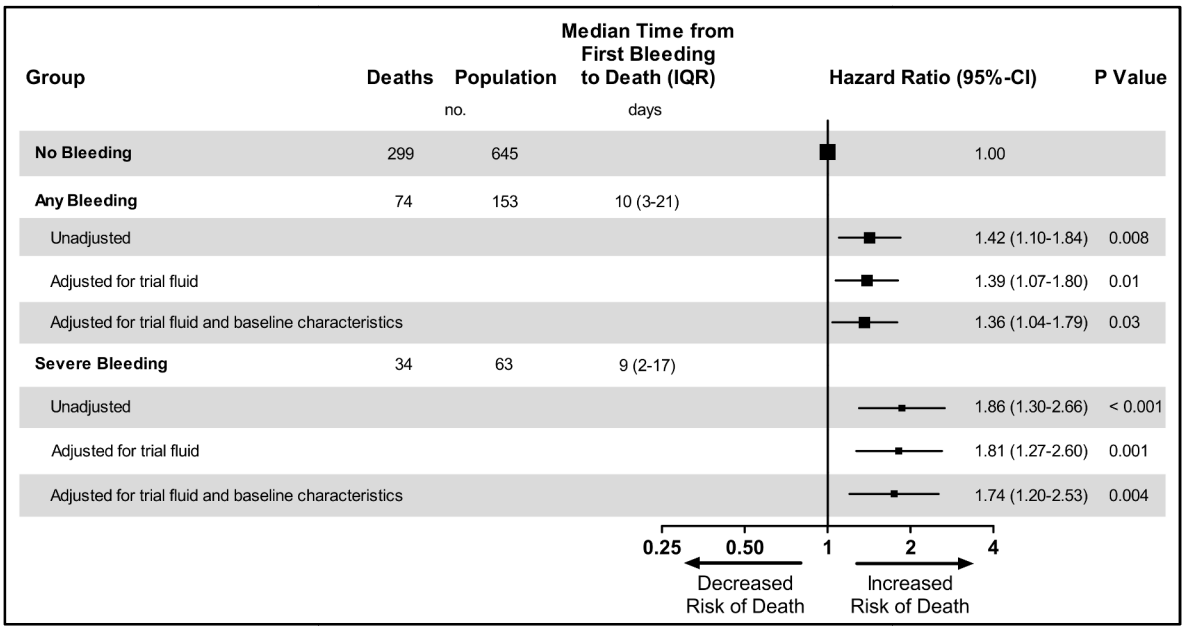

Fig 3 Hazard Ratio for Death According to Occurrence of Bleeding or Severe Bleeding

Cox regression analyses showed a statistically significant increased risk of any bleeding in patients assigned to HES versus Ringer's acetate both in the unadjusted and adjusted analyses (Fig. 2). Hazard ratio estimates for severe bleeding were comparable to those of any bleeding, but were not statistically significant (Fig. 2).

Risk Factors for Bleeding

The clinical characteristics of patients with no bleeding, any bleeding and severe bleeding are shown in Table S3 in the Supplementary Appendix. Admission to a university hospital, surgery prior to randomisation and assignment to fluid resuscitation with HES were all independent risk factors for bleeding (Table 2). For severe bleeding, assignment to HES was no longer an independent risk factor, but baseline platelet count appeared to be so.

Association between Bleeding and Mortality

During the 90 day follow-up period, 373 of the 798 patients (47%) died including 299 of the 645 patients (46%), who did not bleed in the ICU, and 74 of the 153 patients (48%) with any bleeding. Of the 63 patients with severe bleeding, 34 (54%) died. Mortality was highest among patients who stayed in the ICU for a shorter period (Table S4 in the Supplementary Appendix).

The hazard ratio for death was significantly increased among patients with any bleeding and severe bleeding compared to those who did not bleed in the ICU in both unadjusted and adjusted analyses (Fig. 3).

When patients with any bleeding were censored, the hazard ratio estimates for mortality in patients treated with HES versus Ringer's acetate were reduced (Table S5 in the Supplementary Appendix). The censoring of patients with severe bleeding did not change the estimates.

Discussion

In the 6S trial, patients with severe sepsis assigned to 6% HES 130/0.42 versus Ringer's acetate had a markedly increased risk of bleeding, and in multivariate analysis assignment to HES remained an independent risk factor for bleeding. Other risk factors for bleeding appeared to be admission at a university hospital, surgery prior to ICU admission and low platelet count. Most

patients bleed in the first days after randomization when most trial fluid was given. In the same period, INR was higher in the HES group underlining the likely relationship between HES and bleeding.

The increased risk of bleeding with HES was in alignment with reports of several trials in patients undergoing surgery where HES compared to crystalloid or albumin prolonged activated partial thromboplastin time [11], prolonged prothrombin time [12], impaired thrombelastometric/graphic tracings [11–16] and increased blood loss [17]. The Efficacy of Volume Substitution and Insulin Therapy in Severe Sepsis (VISEP) trial and Crystalloid versus Hydroxyethyl Starch Trial (CHEST) found increased use of red blood cell transfusion with HES 200/0.5 and HES 130/0.4, respectively, but bleeding events were not registered in these trials [18, 19]. A smaller trial of HES 130/0.4 did not find any association between HES 130/0.4, bleeding rates and coagulopathy in patients with severe sepsis, which may be due to differences in bleeding registration, less sick patients or lack of statistical power [20]. A recent meta-analysis confirmed the increased use of red blood cells with HES 130/0.4 and HES 130/0.42 in sepsis [21].

Previous studies report that HES affects hemostasis due to hemodilution, but also exerts an additive non-dilutional hemostatic alteration mainly through reduced platelet function and clot strength and affected von Willebrand factor, factor XIII and fibrinogen/fibrin polymerization [13–15, 22–24]. This may explain why we did not detect any significant difference in platelet count between the intervention groups.

The clinical implications of HES induced coagulopathy and bleeding are less clear, but we found a strong association between bleeding and death in the 6S Trial. To determine whether HES induced coagulopathy and bleeding contributed to the overall increased mortality observed with HES, we calculated hazard ratios for death according to trial fluid assignment with censoring of patients with bleeding. We then observed a lower hazard ratio compared to that of the analysis of all patients, which suggested that patients with bleeding contributed to the excess mortality in the HES group. When patients with severe bleeding were censored, the hazard ratio estimates for death with HES remained unchanged, but there were relatively few patients with severe bleeding making these results uncertain.

Our study design cannot prove causality between bleeding and mortality, but a causal relationship is plausible because bleeding may lead to imminent death, but also ischemia and organ injury which later may translate into multiorgan failure and death [25]. In addition, bleeding may lead to red blood cell transfusion which may have late adverse effects [26]. These late mechanisms may have contributed to death in our trial as the time from bleeding to death varied from few days to weeks.

Alternatively, the association between bleeding and death is confounded by other disease processes that increase the risk of death and consequently, bleeding may represent a marker for increased risk of death rather than a cause. Previous findings that HES treatment results in increased inflammation and release of inflammatory mediators [27, 28], which is closely linked to coagulopathy in sepsis [29], support the hypothesis that bleeding is a marker of increased inflammation with HES and not a direct cause of death.

Another explanation is that the longer the patients stayed in the ICU, the higher the risk of death and of being observed with bleeding. In our trial, patients who stayed

longer in the ICU did have higher risk of bleeding, but mortality did not increase correspondingly, so our data do not support this hypothesis.

The strength of our trial was its pragmatic design investigating fluid therapy in clinical practice in high-risk patients in many ICUs with intervention lasting the entire ICU admission and observation lasting beyond ICU discharge to 90 days. Because the trial was adequately powered we were able to inform on patient important outcomes, and the results were less likely affected by imbalance in concomitant interventions.

Our results come with some limitations. Most analyses presented in this paper were planned post-hoc and as such cannot be considered confirmative. Even though we tested multiple outcomes, we refrained from adjusting P values for this type of multiplicity as the likely correlation between outcomes made exact correction impossible. Thus, P values close to 5% should be interpreted with caution. Also, bleeding must have been visible and of a certain volume to be observed clinically, and minor or occult bleeding may not have been detected and registered. We did not observe for bleeding after discharge from the ICU, and we only have limited data describing which part of the coagulation that was affected by HES. Finally, we used potato-derived HES in our trial, which differs in molecular structure from HES derived from maize, but neither clinical nor pre-clinical data provide evidence that the clinical effects of HES depend on its plant source [22, 30].

In conclusion, patient with severe sepsis resuscitated with HES 130/0.42 had increased risk of bleeding which was associated with increased risk of death. HES induced coagulopathy and bleeding may negatively affect outcome in patients with severe sepsis.

Conflicts of interests

AP was the sponsor-investigator of the Scandinavian Starch for Severe Sepsis / Septic Shock (6S) trial and JW and NH were members of the steering committee. The 6S trial was funded by the Danish Research Council, the Rigshospitalet Research Council, and the Scandinavian Society of Anaesthesiology and Intensive Care Medicine (the ACTA Foundation). B Braun Medical delivered trial fluid to all sites free of charge. Neither the funders nor B Braun Medical had an influence on the protocol, trial conduct, data analyses, or reporting of the 6S trial. AP is head of research in his department, which receives research funds from Fresenius Kabi, Germany, Cosmed, Italy, and BioPorto Diagnostics, Denmark. B Braun Medical has covered his travel expenses for presenting 6S trial data at the German Anaesthetic Congress 2012.

PW declares that he has no conflict of interests.

Take-home message

In patient with severe sepsis, treatment with HES increased the risk of bleeding which was associated with increased risk of death. HES induced coagulopathy and bleeding may negatively affect outcome in patients with severe sepsis.

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Supplementary Appendix

Supplement to Haase N et al. Bleeding and Risk of Death with Hydroxyethyl Starch in Severe Sepsis – Post-hoc Analyses of a Randomised Clinical Trial

Contents

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Case record form used daily during the ICU-admission for the registration of bleeding

18 Y N **Intracranial bleeding** as observed on CT- or MR scan

19 **Bleeding episode** as documented in patient files

1 Y N Gastric aspirates as hematemesis, frank blood or "coffee grounds"

2 Y N Stools as melena or frank blood

3 Y N Urine as frank blood

4 Y N Tracheal aspirates as frank blood

5 Y N Wounds

6 Y N During surgery

If YES: documented estimated loss (ml) _____

Table S1. Baseline Characteristics of the Patients[a]

Characteristic	HES 130/0.42 (N=398)	Ringer's acetate (N=400)
Admitted to a university hospital – no. (%)	194 (49)	188 (47)
Septic shock – no. (%)[b]	336 (84)	337 (84)
Active hematologic cancer – no. (%)	36 (9)	36 (9)
Surgery prior to ICU admission – no. (%)[c]	131 (33)	146 (37)
Previous hospital admission for heart failure or myocardial infarction – no. (%)	49 (12)	62 (16)
SAPS II – median (interquartile range)[d]	50 (40-60)	51 (39-62)
SOFA-score – median (interquartile range)[e]	7 (5-9)	7 (5-9)
INR – median (interquartile range)	1.3 (1.2-1.6)	1.3 (1.1-1.6)
Platelet count - 10^9/l		
Median	197	189
Interquartile range	106-275	112-275
Haemoglobin concentration - g/dl		
Median	10.3	10.3
Interquartile range	8.9-11.9	8.9-11.9
Transfusion prior to randomization – no. (%)		
Red blood cells	71 (18)	65 (16)
Fresh frozen plasma	42 (11)	38 (10)
Platelets	25 (6)	22 (6)
Treatment with HES prior to randomization – no. (%)	152 (38)	159 (40)

[a] None of the differences between the two groups were significant ($p<0.05$). The values for the Simplified Acute Physiology Score (SAPS) II, Sepsis-related Organ Failure Assessment (SOFA) score, International normalized ratio (INR), platelet count, haemoglobin concentration, transfusions and treatment with HES pertain to the 24 hours prior to randomization. HES denotes hydroxyethyl starch and ICU intensive care unit.

[b] Septic shock was defined as a mean arterial pressure of less than 70 mm Hg, the need for ongoing treatment with vasopressors or inotropic agents, or a plasma lactate level of more than 4.0 mmol per liter in the hour before randomization.

[c] Surgery includes both elective and emergency surgery prior to the admission on the ICU

[d] SAPS II is calculated from 17 variables; scores range from 0 to 163, with higher scores indicating more severe disease. Data regarding 1 or 2 of the 17 variables were missing for 105 patients in the HES 130/0.42 group and 107 patients in the Ringer's acetate group, so the scores for these patients are not included here.

[e] The SOFA score includes subscores ranging from 0 to 4 for each of five components (circulation, lungs, liver, kidneys, and coagulation). Aggregated scores range from 0 to 20, with higher scores indicating more severe organ failure. The scoring was modified because cerebral failure was not assessed. One of the five subscores was missing for two patients in the HES 130/0.42 group, so their scores are not included here.

Table S2. Risk of Bleeding and Severe Bleeding According to Length of Stay in the Intensive Care Unit[a]

Days in the ICU – no.	Patients in Group – no.	Patients with Bleeding – no. (%)	P Value	Patients with Severe Bleeding – no. (%)	P Value
1 or 2	133	10 (8)	<0.001	5 (4)	<0.001
3 or 4	169	14 (8)		4 (2)	
5 to 8	165	26 (16)		7 (4)	
9 to 15	162	35 (22)		15 (9)	
16 to 89	169	68 (40)		32 (19)	

[a] The patients were divided into five groups of equal size according to their length of stay in the intensive care unit. The P values are from chi-square test. Severe bleeding was defined as intracranial bleeding or bleeding with concomitant transfusion with 3 units of red blood cells.

Table S3. Characteristics of Patients with No Bleeding, Any Bleeding and Severe Bleeding in the ICU[a]

Risk factor	No bleeding (N=645)	Any bleeding (N=153)	Severe bleeding (N=63)
Assigned to HES – no. (%)	305 (47)	93 (61)	37 (59)
Admitted to a university hospital – no. (%)	291 (45)	91 (59)	41 (65)
Surgery prior to ICU admission – no. (%)[b]	206 (32)	71 (46)	33 (52)
Septic shock – no. (%)[c]	538 (83)	135 (88)	55 (87)
Active hematological cancer – no. (%)	53 (8)	19 (12)	9 (14)
SAPS II – median, interquartile range[d]	50 (39-60)	53 (41-63)	53 (42-67)
SOFA score – median, interquartile range[e]	7 (5-9)	8 (6-10)	7 (6-10)
INR – median, interquartile range[f]	1.3 (1.1-1.6)	1.4 (1.2-1.7)	1.4 (1.2-1.8)
Platelet count – 10^9 per liter[g]			
Median	198	154	150
Interquartile range	116-279	81-246	69-244
Treatment with HES prior to randomisation – no. (%)	254 (39)	57 (37)	26 (41)

[a] Severe bleeding was defined as an intracranial bleeding or a bleeding with concomitant transfusion with 3 units of red blood cells. The values for the Simplified Acute Physiology Score (SAPS) II, Sepsis-related Organ Failure Assessment (SOFA) score, International normalized ratio (INR), platelet count, and treatment with HES pertain to the 24 hours prior to randomization. HES denotes hydroxyethyl starch and ICU intensive care unit.

[b] Surgery includes both elective and emergency surgery prior to the admission on the ICU

[c] Septic shock was defined as a mean arterial pressure of less than 70 mm Hg, the need for ongoing treatment with vasopressors or inotropic agents, or a plasma lactate level of more than 4.0 mmol per liter in the hour before randomization.

[d] SAPS II is calculated from 17 variables; scores range from 0 to 163, with higher scores indicating more severe disease. Data regarding 1 or 2 of the 17 variables were missing for 105 patients in the HES 130/0.42 group and 108 patients in the Ringer's acetate group, so the scores for these patients are not included here.

[e] The SOFA score includes subscores ranging from 0 to 4 for each of five components (circulation, lungs, liver, kidneys, and coagulation). Aggregated scores range from 0 to 20, with higher scores indicating more severe organ failure. The scoring was modified because cerebral failure was not

assessed. One of the five subscores was missing for two patients in the HES 130/0.42 group, so their scores are not included here.

[f] INR was missing for 27 patients in the HES 130/0.42 group and 26 patients in the Ringer's acetate group so their values are not included here.

[g] Platelet counts were missing for 2 patients in the HES 130/0.42 group and 2 patients in the Ringer's acetate group so their values are not included here.

Table S4. Risk of Death at 90 Days According to Length of Stay in the Intensive Care Unit[a]

Days in the ICU – no.	Patients in Group – no.	Death at 90 days – no. (%)	P Value
1 or 2	133	83 (62)	0.001
3 or 4	169	68 (40)	
5 to 8	165	68 (41)	
9 to 15	162	72 (44)	
16 to 89	169	82 (49)	

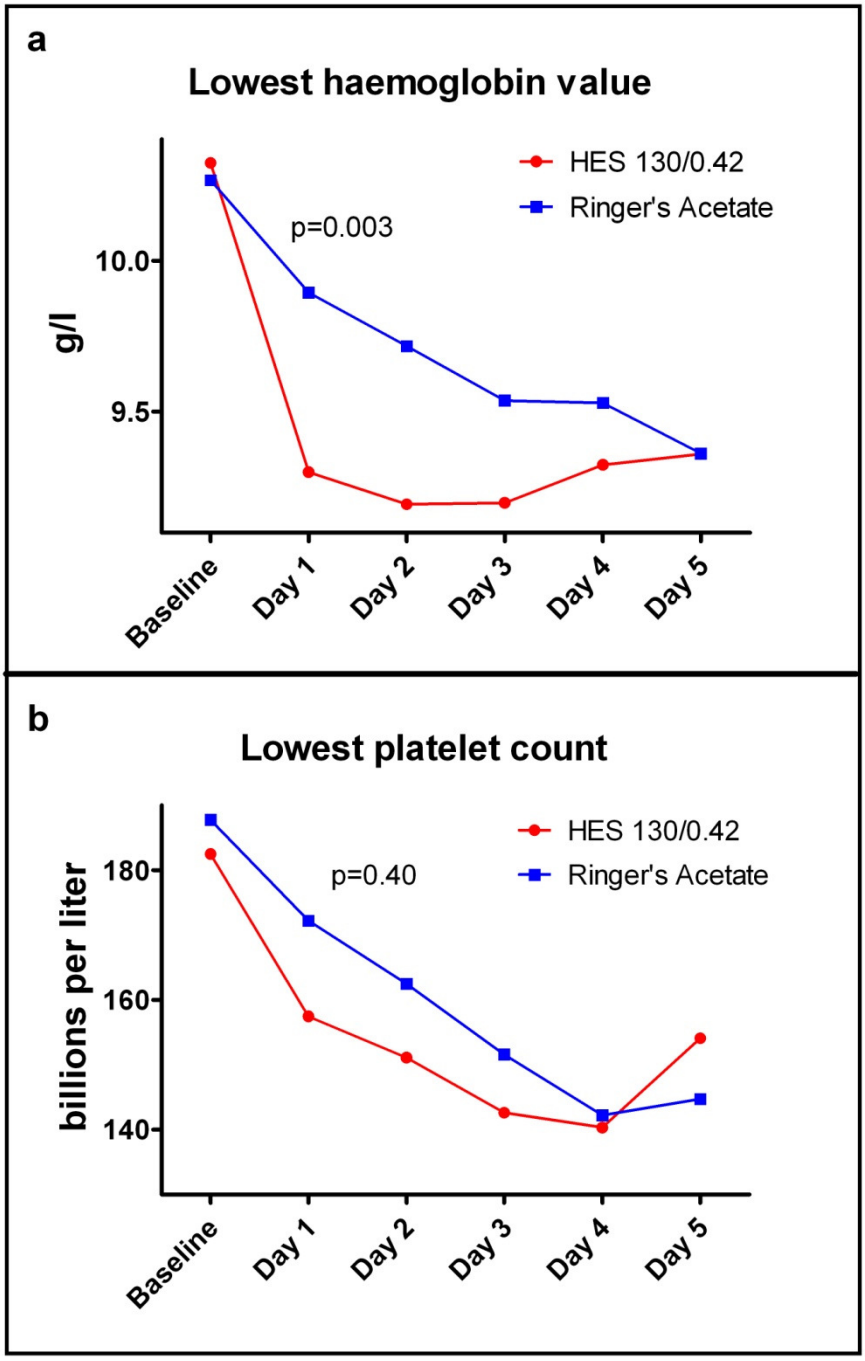
[a] The patients were divided into five groups of equal size according to their length of stay in the intensive care unit. The P value is from chi-square test.

Table S5. Hazard Ratio for Death in Patients treated with HES 130/0.42 vs. Ringer's Acetate, where Patients were censored once they bleed[a]

Type of analysis	Censoring at 90 days		Censoring if any bleeding		Censoring if severe bleeding	
	HR (95%-CI)	P Value	HR (95%-CI)	P Value	HR (95%-CI)	P Value
Unadjusted	1.20 (0.98 to 1.48)	0.074	1.15 (0.91 to 1.44)	0.24	1.20 (0.97 to 1.48)	0.10
Adjusted	1.20 (0.97 to 1.47)	0.09	1.14 (0.90 to 1.44)	0.27	1.18 (0.95 to 1.47)	0.14

[a] In the first column patients were censored at death or 90 days whichever came first. In the second column patients were censored once they developed a bleeding, at death or at 90 days whichever came first. In the third column patients were censored once they developed a severe bleeding, at death or at 90 days whichever came first. Severe bleeding was defined as an intracranial bleeding or a bleeding with concomitant transfusion with 3 units of red blood cells.

Supplementary Figure. Time course of lowest Hemoglobin value (panel a) and lowest Platelet count (panel b) from baseline till five days after Randomization



The curves show median values for each treatment group. P values are for differences in area under the curve. We also examined the time courses using a mixed model adjusted for stratification variables and baseline values. We assumed unstructured covariance and found that both type of trial fluid and time * type of trial fluid significantly associated with hemoglobin levels (P<0.0001 for both), but neither associated with platelet count

PAPER III

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RESEARCH

Hydroxyethyl starch 130/0.38-0.45 versus crystalloid or albumin in patients with sepsis: systematic review with meta-analysis and trial sequential analysis

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Abstract

Objective To assess the effects of fluid therapy with hydroxyethyl starch 130/0.38-0.45 versus crystalloid or albumin on mortality, kidney injury, bleeding, and serious adverse events in patients with sepsis.

Design Systematic review with meta-analyses and trial sequential analyses of randomised clinical trials.

Data sources Cochrane Library, Medline, Embase, Biosis Previews, Science Citation Index Expanded, CINAHL, Current Controlled Trials, Clinicaltrials.gov, and Centerwatch to September 2012; hand search of reference lists and other systematic reviews; contact with authors and relevant pharmaceutical companies.

Study selection Eligible trials were randomised clinical trials comparing hydroxyethyl starch 130/0.38-0.45 with either crystalloid or human albumin in patients with sepsis. Published and unpublished trials were included irrespective of language and predefined outcomes.

Data extraction Two reviewers independently assessed studies for inclusion and extracted data on methods, interventions, outcomes, and risk of bias. Risk ratios and mean differences with 95% confidence intervals were estimated with fixed and random effects models.

Results Nine trials that randomised 3456 patients with sepsis were included. Overall, hydroxyethyl starch 130/0.38-0.45 versus crystalloid or albumin did not affect the relative risk of death (1.04, 95% confidence interval 0.89 to 1.22, 3414 patients, eight trials), but in the predefined analysis of trials with low risk of bias the relative risk of death was 1.11 (1.00 to 1.23, trial sequential analysis (TSA) adjusted 95% confidence interval 0.95 to 1.29, 3016 patients, four trials). In the hydroxyethyl starch group, renal replacement therapy was used more (1.36, 1.08 to 1.72, TSA adjusted 1.03 to 1.80, 1311 patients, five trials), and the relative risk of acute kidney injury was 1.18 (0.99 to 1.40, TSA adjusted 0.90 to 1.54, 994 patients, four trials). More patients in the hydroxyethyl starch group were transfused with red blood cells (1.29, 1.13 to 1.48, TSA adjusted 1.10 to 1.51, 973 patients, three trials), and more patients had serious adverse events (1.30, 1.02 to 1.67, TSA adjusted 0.93 to 1.83, 1069 patients, four trials). The transfused volume of red blood cells did not differ between the groups (mean difference 65 mL, 95% confidence interval –20 to 149 mL, three trials).

Conclusion In conventional meta-analyses including recent trial data, hydroxyethyl starch 130/0.38-0.45 versus crystalloid or albumin increased the use of renal replacement therapy and transfusion with red blood cells, and resulted in more serious adverse events in patients with sepsis. It seems unlikely that hydroxyethyl starch 130/0.38-0.45 provides overall clinical benefit for patients with sepsis.

Introduction

Colloids are used more often for resuscitation in the intensive care unit than crystalloids. The choice of colloid varies noticeably between countries, but worldwide hydroxyethyl starch is most commonly used and thus more used than, for example, human albumin and gelatin.[1] The use of hydroxyethyl starch is controversial as the former higher molecular weight hydroxyethyl starch 200/0.5-0.6 caused acute kidney injury in two randomised clinical trials of patients with sepsis.[2 3] The newer starches with molecular weights of 130 kDa and substitution ratios ranging from 0.38 to 0.45 have been claimed to be safer, but the data to support this are insufficient.[4] Owing to the lack of data on hydroxyethyl starch 130/0.38-0.45, previous systematic reviews have been inconclusive about the

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Extra material supplied by the author (see http://www.bmj.com/content/346/bmj.f839?tab=related#webextra)

Search strategy, details of included studies, and figures
Reasons for exclusion of trials

benefits and harms of this colloid compared with other fluids.[4-8] The recent publication of three large trials comparing hydroxyethyl starch 130/0.38-0.45 with crystalloids in patients with sepsis calls for an updated systematic review to inform on the benefits and harms of this colloid in patients with sepsis, which is highly needed as fluid alternatives are available.[9-11]

We assessed the effects of hydroxyethyl starch 130/0.38-0.45 versus crystalloids or human albumin on all cause mortality, kidney injury, bleeding, and serious adverse events in patients with sepsis.

Methods

This systematic review is based on the methodology recommended by the Cochrane Collaboration.[12] The protocol was published in the PROSPERO register (www.crd.york.ac.uk/PROSPERO) before the literature search.

Eligibility criteria

Potentially eligible trials had to be prospective and randomised, include patients with sepsis, have one intervention group that received hydroxyethyl starch 130 with substitution ratios between 0.38 and 0.45 in any concentration and in any carrier solution, and have at least one other intervention group that received either crystalloid or human albumin.

We included trials irrespective of language, publication status, patient's age, indication for fluid therapy, and predefined outcomes. If the patients with sepsis constituted a subgroup of the trial population, we included the trial only if the randomisation was stratified for the presence of sepsis or if the population with sepsis was larger than 500 participants. We also included quasirandomised and observational studies with more than 500 patients receiving hydroxyethyl starch 130/0.38-0.45, but evaluated these for serious adverse events only. Exclusion criteria were studies in animals, patients without sepsis, hydroxyethyl starch products of other molecular weights or substitution ratios, crossover studies, and studies comparing hydroxyethyl starch with other synthetic colloid solutions.

Search strategy

We searched the Cochrane central register of controlled trials, Medline, Embase, Biosis Previews, Science Citation Index Expanded, and Cumulative Index to Nursing and Allied Health Literature. As hydroxyethyl starch 130/0.38-0.45 was introduced on the market in 1999 we limited the search to references from 1995 or later. We also hand searched the reference lists of included trials and other systematic reviews of fluid therapy for further trials.

Unpublished trials were sought through trial registries (www.controlled-trials.com, www.clinicaltrials.gov, and www.centerwatch.com), and we contacted relevant pharmaceutical companies for unpublished data. The electronic literature search was last updated 10 September 2012. See the supplementary file for details of the search, including the search string.

Study selection

Two authors (NH, LIH, BL, or MW) independently reviewed all titles and abstracts identified in the literature search and excluded trials that were obviously not relevant. The remaining trials were evaluated in full text. Disagreements were resolved with JW.

Data extraction

Two authors (NH, LIH) independently extracted information from each included trial by using a pre-made data extraction form. The extracted information included trial characteristics (single or multicentre and country), characteristics of the trial participants (age, sex, and disease severity), criteria for inclusion and exclusion, type of intervention (indication, dosing, duration, and comparator fluid), and outcomes.

The predefined primary outcomes of this review were overall mortality and number of patients still receiving renal replacement therapy at the maximum length of follow-up. The predefined secondary outcomes were the number of patients receiving renal replacement therapy at any time during the follow-up period, number of patients having acute kidney injury, number of patients receiving red blood cell transfusion, total volume of red blood cells transfused, number of patients having a bleeding episode, estimated blood loss, and number of patients having one or more serious adverse events. We contacted the corresponding authors for data on outcomes that were not reported in their publications.

Translators extracted data from all relevant non-English articles.

Risk of bias assessment

To determine the validity of the included trials, we assessed the risk of bias as advised by the Cochrane Collaboration,[12] including the domains of random sequence generation, allocation concealment, blinding, incomplete outcome data, selective outcome reporting, baseline imbalance, bias due to vested financial interest, and academic bias. If one or more domains were judged as being high or unclear, we classified the trial as having a high risk of bias. Since the need for fluids is difficult to assess objectively, the choice to give fluid instead of vasopressors or inotropes may depend on the expected potency of the fluid. Thus, unblinding may lead to systematic differences in interventions or cointerventions between the intervention groups, so we classified all unblinded trials as being at high risk of bias for all outcomes including mortality unless study fluids were given in fixed doses.

Statistical analysis

Review Manager 5.1.6 was used for statistical analyses, and we used the TSA program version 0.9 beta (www.ctu.dk/tsa) for trial sequential analyses. For each included trial we calculated the relative risks (95% confidence intervals) for dichotomous outcomes and risk difference (95% confidence intervals) for continuous outcomes, and we pooled these measures in meta-analyses.

Heterogeneity among trials was quantified with inconsistency factor (I^2) statistics. If the I^2 statistic was 0, we reported the results from a fixed effect model. If the I^2 statistic was greater than 0, we reported the results from both random effects and fixed effects models.

Sensitivity analyses included application of continuity correction in trials of zero events[13] and exclusion of the smallest trial, the largest trial, and trials financed by industry.

We did a predefined subgroup analysis with stratification of trials according to risk of bias. To further explore possible reasons for a high or moderate statistical heterogeneity we did an explorative post hoc subgroup analysis stratifying trials according to length of follow-up.

Some authors have suggested that conventional meta-analysis should not be trusted without further evaluation, as cumulative meta-analyses of trials are at risk of producing random errors

because of sparse data and repetitive testing of accumulating data.[14 15] We therefore challenged the meta-analyses with the application of trial sequential analysis—a sensitivity analysis that widens the confidence intervals in case the data are too sparse to draw firm conclusions. Trial sequential analysis is similar to interim analysis in a single trial where the monitoring boundaries are used to decide whether the P value is sufficiently small to show the anticipated effect and whether the trial should be terminated early. In the same manner, trial sequential monitoring boundaries can be applied to meta-analyses.[14-17]

Trial sequential analysis depends on the quantification of the required information size (the meta-analysis sample size). We calculated a diversity, D^2, adjusted required information size since the heterogeneity adjustment with I^2 underestimate the required information size.[18] We did the trial sequential analysis with the intention to maintain an overall 5% risk of a type I error and a power of 80%. For the calculation of the required information size we anticipated an intervention effect of a 20% relative risk increase. For renal replacement therapy, bleedings, and serious adverse events we used an anticipated effect of 35%, since we expected a much lower event proportion for these outcomes. For mortality, we observed only an 11% relative risk increase in trials with low risk of bias and used this effect instead in the trial sequential analysis of mortality. We provide the 95% confidence intervals adjusted for sparse data and repetitive testing, which we describe as the trial sequential analysis adjusted 95% confidence intervals.

Results

Figure 1⇓ summarises the results of the search. The main reasons for exclusion of randomised trials were that the patients did not have sepsis and the trials evaluated a hydroxyethyl starch solution other than hydroxyethyl starch 130/0.38-0.45 (see supplementary table).[19-50] No language restrictions were applied; one paper was in Spanish, one in Japanese, four in Russian, and four in Chinese. Overall nine trials met the inclusion criteria.[9-11 51-56] One trial was still unpublished.[51] The authors of six trials were successfully contacted[9-11 51 53 54] and data were obtained for eight.[9-11 51 53-56] A Chinese researcher extracted data from two trials published in Chinese.[55 56] All other trials were published in English. No observational study was identified with more than 500 patients with sepsis receiving hydroxyethyl starch 130/0.38-0.45 to evaluate for adverse events.

Characteristics of trials

The four largest trials were blinded and had long term (>28 days) follow-up.[9-11 51] The remaining trials were either unblinded, had unclear methodology, or had shorter follow-up times (≤28 days). Table 1⇓ shows the characteristics of the included trials, and table 2⇓ the observation period for each outcome.

Participants

The included trials enrolled 3456 adults with sepsis on an intensive care unit. One trial included a broad spectrum of patients on the intensive care unit, but in this review only the subgroup of patients with sepsis were included.[10] All but two trials included patients with both sepsis and organ failure (severe sepsis).[10 51] The definitions of organ failure varied slightly between trials, but in most included various clinical signs of hypoperfusion as, for example, oliguria, hypotension, and increased lactate levels. Only one trial specifically stated that all patients had septic shock.[55]

Interventions

The type of hydroxyethyl starch studied was 6% Voluven (hydroxyethyl starch 130/0.4 (range 0.38-0.45) in saline, Fresenius Kabi, Bad Homburg, Germany) in six trials,[10 11 51-54] 6% Tetraspan (hydroxyethyl starch 130/0.42 (range 0.40-0.44) in Ringer's acetate, B Braun Melsungen, Melsungen, Germany) in one trial,[9] and 6% hydroxyethyl starch 130/0.4 without a statement of the brand name in two trials.[55 56] Two trials compared starch with human albumin 20%,[52 53] whereas the remaining trials used crystalloid as comparator. In one study two groups received hydroxyethyl starch 130/0.4 in isotonic saline or hypertonic saline.[56] These groups were pooled and compared with the third group receiving Ringer's lactate.

Trial fluid was used for resuscitation in eight trials[9-11 51 52 54-56] and given as fixed doses in one trial.[53] The duration of the intervention varied from 24 hours to the entire stay on the intensive care unit to a maximum of 90 days. Cumulative doses of hydroxyethyl starch ranged from 2.1 litres to 6.4 litres with no obvious relation between duration of intervention and total dose.

Bias risk assessment

The risk of bias could be fully judged in six trials.[9-11 51 53 54] Four of these were judged to be of low risk of bias in all domains,[9 10 51 53] the fifth was sponsored by industry and had potential academic bias,[11] and the sixth had a high risk of bias owing to lack of blinding.[54]

In the remaining three trials at least one domain was judged to be unclear, but all of these trials were judged to be of high risk of bias in other domains (table 3⇓, also see the supplementary file).

Clinical outcomes

All cause mortality

Mortality data were obtained from eight trials including 3414 patients.[9-11 51 53-56] The observation period in four of these trials (3156 patients) was longer than 28 days (table 2).[9-11 51] The meta-analysis of all eight trials showed no significant difference in mortality in patients treated with hydroxyethyl starch 130/0.38-0.45 compared with crystalloid or albumin (random effects: relative risk 1.04, 95% confidence interval 0.89 to 1.22; P=0.64; fixed effect: 1.08, 0.98 to 1.19; P=0.13; I^2=37%; fig 2⇓). The trial sequential analysis adjusted 95% confidence interval was 0.70 to 1.54 (see supplementary file). The predefined analysis of trials with low risk of bias showed a relative risk of 1.11 (1.00 to 1.23; P=0.05; I^2=0%), but the test for subgroup difference between trials with low versus high risk of bias was not significant (P=0.13, fig 2). Trial sequential analysis of trials with low risk of bias showed that 3016 of the required information size of 6237 patients was accrued. The cumulative z curve touched the conventional boundary for harm but did not cross the trial sequential monitoring boundary for harm (trial sequential analysis adjusted 95% confidence interval of trials with low risk of bias 0.95 to 1.29) (fig 3⇓). However, the z curve will need to pass through the futility area to reach the area of benefit, leaving little chance that hydroxyethyl starch will turn out to reduce the relative risk of death with 11% if further trials are conducted in patients with sepsis.

The post hoc subgroup analysis according to time of follow-up showed a significant increase in all cause mortality in trials with follow-up for more than 28 days (relative risk 1.11, 95% confidence interval 1.01 to 1.22; P=0.04; I^2=0%) versus a non-significant decrease in all cause mortality in trials with

follow-up for 28 days or less (0.63, 0.35 to 1.15; P=0.13). The test for subgroup differences was not significant at the 5% level (P=0.07, see supplementary file). The trial sequential analysis adjusted 95% confidence intervals of trials with follow-up for more than 28 days was 0.95 to 1.29 (see supplementary file).

Renal replacement therapy at end of follow-up

Five trials had data on renal replacement therapy, with observation periods ranging from 24 hours to one year.[9 11 51 53 54] The Scandinavian Starch for Severe Sepsis/Septic Shock (6S) trial reported that two patients—one in each intervention group—were still being treated with renal replacement therapy at the end of follow-up.[9] In Basel Starch Evaluation in Sepsis (BaSES)[51] no patient required renal replacement therapy after one year, and in the trial by Guidet et al (CRYSTMAS)[11] one patient in the hydroxyethyl starch group was treated with renal replacement therapy for more than 28 days, but it was unclear whether this lasted until end of follow-up. These data did not undergo meta-analysis.

Renal replacement therapy at anytime during follow-up

The same five trials had data on the number of patients treated with renal replacement therapy at anytime during follow-up. One trial had zero events in three days.[53] The pooled analysis showed that patients receiving hydroxyethyl starch 130/0.38-0.45 had a significantly increased risk of receiving renal replacement therapy (relative risk 1.36, 95% confidence interval 1.08 to 1.72; P=0.009; I^2=0%; fig 4⇓). Application of an empirical continuity correction of 0.01 in the no event trial did not change the result. Trial sequential analysis showed that 1311 of the required information size of 1654 patients was accrued, but the cumulative z curve crossed the trial sequential monitoring boundary for harm providing firm evidence of increased use of renal replacement therapy in patients treated with hydroxyethyl starch compared with crystalloid or albumin (trial sequential analysis adjusted 95% confidence interval 1.03 to 1.80) (fig 5⇓).

Acute kidney injury

Acute kidney injury was defined as a twofold increase of serum creatinine levels during the observation period, as this was consistently reported in the four trials with data on kidney function.[9 11 53 54] The observation periods ranged from 24 hours to the entire stay on the intensive care unit. One trial had no events,[53] and the pooled analysis of the remaining three trials showed a non-significant increase in the risk of acute kidney injury in the hydroxyethyl starch group (relative risk 1.18, 95% confidence interval 0.99 to 1.40; P=0.07; I^2=0%) (see supplementary file). Application of an empirical continuity correction of 0.01 in the no event trial did not change the result. The trial sequential analysis adjusted 95% confidence interval was 0.90 to 1.54 (see supplementary file).

Transfusions with red blood cells, bleeding, and blood loss

Three trials provided data on transfusions, with observation periods ranging from 24 hours to the entire stay on the intensive care unit.[9 11 54] The risk of being transfused with red blood cells was significantly higher in the hydroxyethyl starch group (1.29, 95% confidence interval 1.13 to 1.48; P<0.001; I^2=0%) (see supplementary file). The trial sequential analysis adjusted 95% confidence interval was 1.10 to 1.51, providing firm evidence for an increased risk of transfusion with red blood cells if treated with hydroxyethyl starch 130/0.38-0.45 (see supplementary file).

The mean volume of red blood cells did not differ between the groups (mean difference 65 mL, 95% confidence interval −20 to 149 mL; P=0.13; I^2=0%) (see supplementary file).

The number of patients having at least one bleeding episode (relative risk 1.34, 95% confidence interval 0.81 to 2.21; P=0.26; I^2=38%) and blood loss (mean difference 26 mL, −89 to 140 mL; P=0.66; I^2=0%) were reported in two trials (see supplementary file).[9 11]

Serious adverse events

Four trials reported serious adverse events, two of which registered these during the entire stay on the intensive care unit.[9 11 53 54] In the 6S trial serious adverse events were restricted to severe bleeding and severe allergic reactions,[9] whereas CRYSTMAS used broad criteria.[11] The last two trials did not specify the definition of serious adverse events, and one of them had zero events in 24 hours follow-up.[54] According to the good clinical practice guidelines by the International Conference on Harmonisation, death should count as a serious adverse event in the analysis,[57] but we were unable to get the composite endpoint of either death or serious adverse events from more than one trial.[9]

The pooled analysis of the three trials showed a significantly increased risk of serious adverse events with hydroxyethyl starch 130/0.38-0.45 (relative risk 1.30, 95% confidence interval 1.02 to 1.67; P=0.03; I^2=0%) (see supplementary file). The application of a continuity correction to the zero event trial neither changed the estimate nor the confidence interval. The trial sequential analysis adjusted 95% confidence interval was 0.93 to 1.83 (see supplementary file).

Discussion

The main finding of this systematic review was that patients assigned to hydroxyethyl starch 130/0.38-0.45 had in conventional meta-analysis a statistically significant increased risk of getting renal replacement therapy, transfusion with red blood cells, and serious adverse events. The recent large, well designed trials showed consistent results with no statistical heterogeneity and the findings are likely to be confirmed when further data of the patients with sepsis in the Crystalloid versus Hydroxyethyl Starch (CHEST) trial[10] become available, since the hydroxyethyl starch group in this trial had more use of renal replacement therapy and transfusion with red blood cells and more serious adverse events.

The pooled analysis of mortality showed neither benefit nor harm, but trials with a low risk of bias suggested an excess mortality of 11%. In addition, our post hoc analysis of trials with follow-up for more than 28 days showed increased mortality. Thus the pooled analysis of mortality may be influenced by trials of poor quality and too short follow-up, making interpretation difficult.

The sensitivity analysis with trial sequential analysis widened the confidence intervals of the conventional meta-analyses when data were too sparse to draw firm conclusions. With this strict approach the increased risk of renal replacement therapy and transfusion with red blood cells remained statistically significant. For mortality in trials with low risk of bias and long term follow-up, trial sequential analysis indicated a lack of statistical significance for increased mortality, but also that it is unlikely that hydroxyethyl starch will result in a relative mortality

reduction of 11% if further trials are conducted in patients with sepsis.

Our results are consistent with the fact that a high fraction of hydroxyethyl starch 130/0.38-0.45 is deposited in the tissues where it cannot be metabolised[58] and may act as a foreign body with long term toxic effects, which have been described in the kidney, liver, and bone marrow.[59-61] In addition, the use of renal replacement therapy has repeatedly been associated with death.[62 63] Our findings are in alignment with the results of two sepsis trials of hydroxyethyl starch 200/0.5-0.6 on renal impairment and late adverse effects.[2 3] Thus the adverse effects of hydroxyethyl starch may be a class effect independent of molecular weight and substitution ratio.

Some hypothesise that bad outcome in patients treated with hydroxyethyl starch is due to inappropriate dosing, including the lack of predefined triggers and goals for fluid resuscitation. No data currently support this belief, as there was no suggestion of an overall favourable outcome in any trial with adequate bias control and follow-up—not even in the trial designed by one of the manufacturers of hydroxyethyl starch.[11]

Strengths and limitations of the review

The compliance with the recommendations of the Cochrane Collaboration is a major strength of our systematic review. This included a prepublished protocol, an up to date extensive literature search with no language restrictions, independent screening of all references by two authors, inclusion of trials irrespective of publication and language status and reported outcomes, independent data extraction by two authors, bias risk assessment, and contact with the corresponding authors of the included trials for additional information. In addition, we reduced the risk of random error in the meta-analyses with the application of trial sequential analysis using predefined variables to increase the robustness of this analysis.

We excluded trials comparing hydroxyethyl starch with other synthetic colloids that may possess the same harmful effects and thereby mask any adverse effects of hydroxyethyl starch.[64] To get a clinical applicable result, we restricted the review to hydroxyethyl starch 130/0.38-0.45 as clinicians almost exclusively use these starches. Including all types of hydroxyethyl starch in the analysis would probably have resulted in a stronger group difference instead.

The post hoc subgroup analysis of mortality in trials according to length of follow-up might have resulted in spurious findings. In general, however, some adverse effects undoubtedly develop slowly, and if the observation period is too short, such events may not be captured. In the largest trials of hydroxyethyl starch in sepsis the relative risk of death increased from day 28 to day 90, indicating that the observation period for mortality should be longer than 28 days, and this was the rationale for our subgroup analysis.[2 9 10]

We chose to include trials with either crystalloid or albumin solutions as comparators as no adverse effects were seen with albumin versus saline in patients with severe sepsis in a large intensive care unit trial.[65] However, most of the included trials compared hydroxyethyl starch with a crystalloid, and this may prevent us from drawing firm conclusions on the effects of albumin. Neither can this review tell whether patients other than those with sepsis may experience adverse effects from hydroxyethyl starch, but the CHEST trial found increased serious adverse events and use of renal replacement therapy with hydroxyethyl starch in a broad population of intensive care unit patients, suggesting adverse effects beyond those seen in sepsis.

Additional limitations of this review are due to bias of the included trials, inadequate follow-up, and trials not reporting all the outcome measures. The definitions of serious adverse events were heterogeneous, so the group difference should be interpreted with caution. The RIFLE (Risk of renal dysfunction, Injury to the kidney, Failure of kidney function, Loss of kidney function and End-stage renal disease)[66] and AKIN (Acute Kidney Injury Network)[67] classifications may be better measures for acute kidney injury, but we used renal replacement therapy and doubling of creatinine levels instead as these more simple outcomes were more often reported.

Relation to other reviews and implication for future research

Several well conducted systematic reviews have been published on hydroxyethyl starch 130/0.38-0.45[4 8] and on hydroxyethyl starch and fluid therapy in general.[5-7] Owing to the previous lack of data on hydroxyethyl starch 130/0.38-0.45, these reviews have been inconclusive about the benefit and harm of hydroxyethyl starch 130/0.38-0.45 compared with other fluids. In comparison, this review contains data from new large trials and applies trial sequential analysis on the results.

Hydroxyethyl starch 130/0.38-0.45 is often used in the surgical setting and may continue despite the raised safety issues in patients with sepsis. If use does continue, then well powered surgical trials are urgently needed to ensure the safety of patients.

Conclusion

In conventional meta-analyses including recent trial data, hydroxyethyl starch 130/0.38-0.45 versus crystalloid or albumin in patients with sepsis was associated with an increased use of renal replacement therapy and transfusion with red blood cells and more serious adverse events. The pooled analysis of mortality showed no group difference, but this analysis may be influenced by trials of low quality. After trial sequential analysis adjustment for sparse data and multiple updating in cumulative meta-analysis it seems unlikely that hydroxyethyl starch provides overall clinical benefit for patients with sepsis.

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Contributors: NH developed the protocol, was responsible for the searches, selected trials, extracted data, assessed the risk of bias of trials, did the data analysis, and developed the final review. AP developed the protocol, analysed data, and developed the final review. LIH developed the protocol, selected trials, extracted data, assessed the risk of bias of trials, and developed the final review. MS extracted data, assessed the risk of bias of trials, and developed the final review. BL and MW developed the protocol, selected trials, and developed the final review. JW developed the initial idea for the review, developed the protocol, selected trials, advised on statistical methods, analysed data, and developed the final review. All authors read and approved the final manuscript. NH and JW are the guarantors.

Competing interests: All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: AP was principal

What is already known on this topic

Hydroxyethyl starches (HES) with molecular weights of 130 kDa and substitution ratios ranging from 0.38 to 0.45 are the most commonly used colloids wordwide, but their safety and efficacy have not been established in patients with sepsis

Owing to lack of data, previous systematic reviews on HES 130/0.38-0.45 and on HES in general have been inconclusive about the benefits and harms of HES compared with other fluids

What this study adds

This systematic review includes the results of four recent randomised clinical trials of HES 130/0.38-0.45 comprising more than 3000 patients with sepsis

The pooled analysis of trials showed that treatment with HES increased the risk of having renal replacement therapy, red blood cell transfusion, and severe adverse reactions

It seems unlikely therefore that HES provides overall clinical benefit for patients with sepsis

investigator for the Scandinavian Starch for Severe Sepsis/Septic Shock (6S) trial and NH and JW were members of the steering committee. The 6S trial was funded by the Danish Research Council, the Rigshospitalet Research Council, and the Scandinavian Society of Anaesthesiology and Intensive Care Medicine (the ACTA foundation). B Braun Melsungen delivered trial fluid to all sites free of charge. Neither the funders nor B Braun Melsungen had an influence on the protocol, trial conduct, data analyses, or reporting of the 6S trial. AP is head of research in his intensive care unit, which receives research funds from Fresenius Kabi and BioPorto. B Braun Melsungen has covered his travel expenses for presenting 6S data at the German Anaesthetic Congress 2012. MS was principal investigator for the Basel starch evaluation in sepsis (BaSES) trial. The BaSES trial was funded by the Department of Anaesthesia and Intensive Care of the University Hospital Basel. Fresenius Kabi delivered study fluids for free and paid for the packaging and blinding process. A signed contract between Fresenius Kabi and MS stated that MS was free to publish all data without influence from Fresenius Kabi. Fresenius Kabi covered travel expenses for MS's participation in meetings about fluid resuscitation.

Ethical approval: Not required.

Data sharing: No additional data available.

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Tables

Table 1| Characteristics of included studies

Trial	No of patients	Centre status, setting	Blinding	No of intervention groups	Diagnostic group	Indication for intervention	HES solution	Comparator	Intervention period	Total dose of HES	Contact with authors successful
6S[9]	804	Multicentre, ICU	Yes	2	Severe sepsis	Resuscitation	6% Tetraspan*	Ringer's acetate	ICU stay. Maximum 90 days	Median 3000 (IQR 1507-5100)	Yes
BaSES[51]	241	Two ICUs in one hospital	Yes	2	Sepsis	Resuscitation	6% Voluven†	Isotonic saline	5 days	Median 3775 (IQR 2018-6347)	Yes
CHEST[10]	1937	Multicentre, ICU	Yes	2	Sepsis	Resuscitation	6% Voluven†	Isotonic saline	ICU stay. Maximum 90 days	Mean 2104 (SD 850‡)	Yes
CRYSTMAS[11]	196	Multicentre, ICU	Yes	2	Severe sepsis	Resuscitation	6% Voluven†	Isotonic saline	4 days	Mean 2615 (SD 1499)	Yes
Dolecek 2009[53]	56	Single, ICU	No	2	Severe sepsis	Fixed dose	6% Voluven†	Albumin 20%	3 days	4×250 mL/day in 3 days	Yes
Dubin 2010[54]	25	Multicentre, ICU	No	2	Sepsis and tissue hypoperfusion	Resuscitation	6% Voluven†	Isotonic saline	24 hours	Mean 2610 (SD 885)	Yes
Lv 2012[55]	42	Single, ICU	Unclear	2	Septic shock	Resuscitation	Unclear	Ringer's lactate	24 hours	Mean 2770 (SD 590)	No
Palumbo 2006[52]	20	Single, ICU	No	2	Severe sepsis	Maintenance of pulmonary capillary wedge pressure	6% Voluven†	Albumin 20%	Unclear	No information on doses	No
Zhu 2011[56]	135	Single, ICU	No	3	Severe sepsis	Resuscitation	6% HES 130/0.4 (unclear brand)	Ringer's lactate	24 hours	HES+hypertonic saline group: mean 5475 (SD 209), HES group: mean 6383 (SD 287)	No

HES=hydroxyethyl starch; ICU=intensive care unit; IQR=interquartile range; SD=standard deviation.

*6% hydroxyethyl starch 130/0.42 in Ringer's acetate (B Braun Melsungen, Melsungen, Germany).

†6% hydroxyethyl starch 130/0.4 in saline (Fresenius Kabi, Bad Homburg, Germany).

‡Only reported for first four days.

Table 2| Observation period for outcomes

Trial	Mortality	Renal replacement therapy	Acute kidney injury	Red blood cell transfusion	Bleeding and blood loss	Serious adverse events
6S[9]	90 days	90 days	ICU	ICU	ICU	ICU
BaSES[51]	1 year	1 year	—	—	—	—
CHEST[10]	90 days	—	—	—	—	—
CRYSTMAS[11]	90 days	ICU	ICU	ICU	4/8 days	ICU
Dolecek 2009[53]	28 days	72 hours	72 hours	—	—	72 hours
Dubin 2010[54]	28 days	24 hours	24 hours	24 hours	—	24 hours
Lv 2012[55]	Unclear*	—	—	—	—	—
Palumbo 2006[52]	—	—	—	—	—	—
Zhu 2011[56]	24 hours	—	—	—	—	—

ICU=intensive care unit.

*Death in hospital or ICU, although not specifically stated.

Table 3| **Risk of bias**

Trial	Random sequence generation	Allocation concealment	Blinding	Incomplete outcome data	Selective outcome reporting	Baseline imbalance	Vested financial interests	Academic bias
6S[9]	Low	Low	Low	Low	Low	Low	Low	Low
BaSES[51]	Low	Low	Low	Low	Low	Low	Low	Low
CHEST[10]	Low	Low	Low	Low	Low	Low	Low	Low
CRYSTMAS[11]	Low	Low	Low	Low	Low	Low	High	High
Dolecek 2009[53]	Low	Low	Low	Low	Low	Low	Low	Low
Dubin 2010[54]	Low	Low	High	Low	Low	Low	Low	Low
Lv 2012[55]	Low	High	Unclear	Unclear	Low	Low	Unclear	Unclear
Palumbo 2006[52]	Unclear	High	Unclear	Low	High	Low	Unclear	Low
Zhu 2011[56]	Unclear	High	High	Unclear	Low	Low	Low	Unclear

See supplementary file to support judgment.

Figures

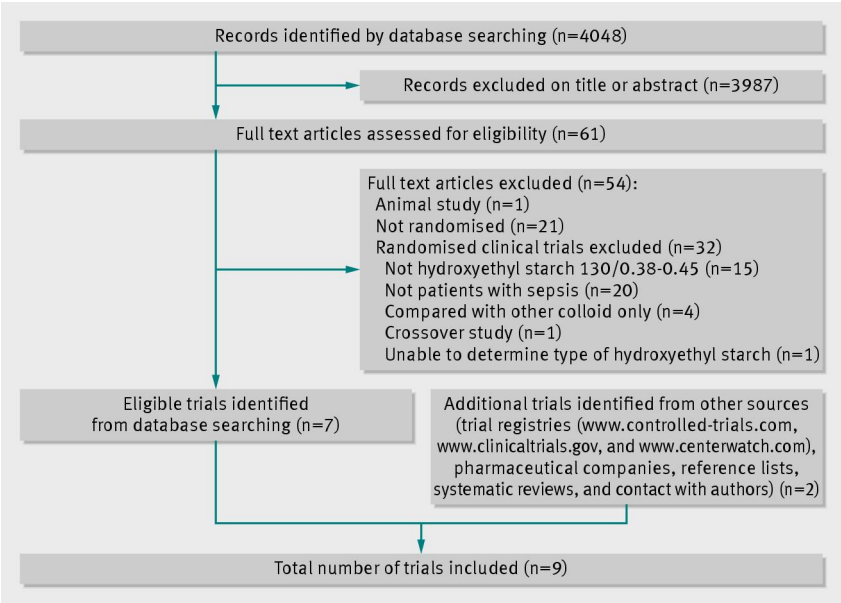


Fig 1 Flow of papers through review. Each of the 32 excluded randomised clinical trials may have more than one reason for exclusion

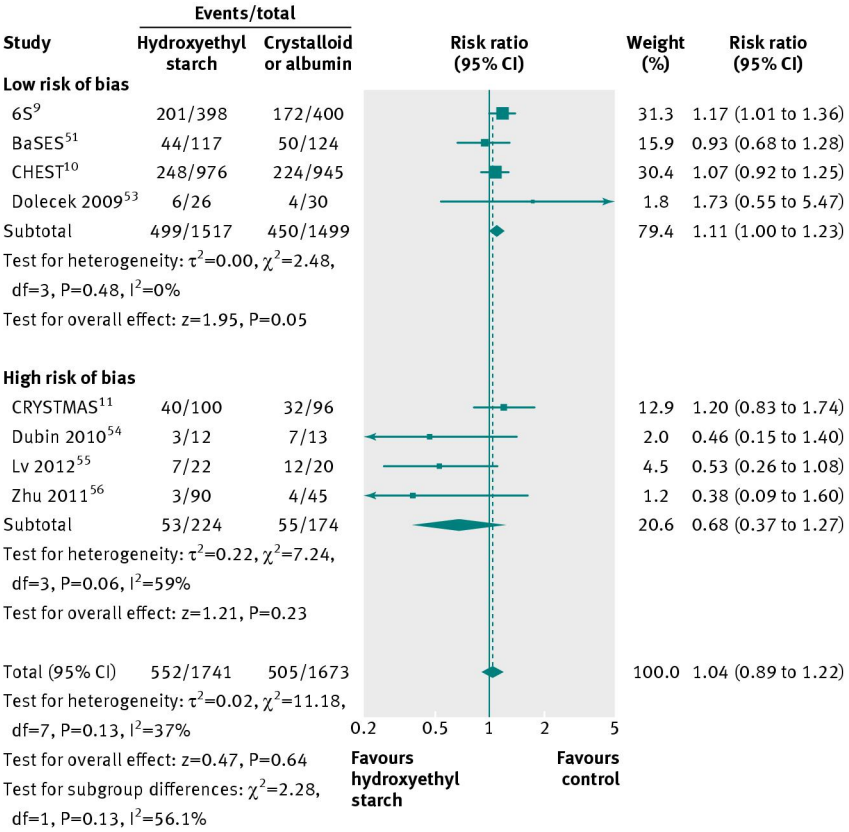


Fig 2 Forest plot of all cause mortality in relation to risk of bias in trials. Size of squares for risk ratio reflects weight of trial in pooled analyses. Horizontal bars represent 95% confidence intervals

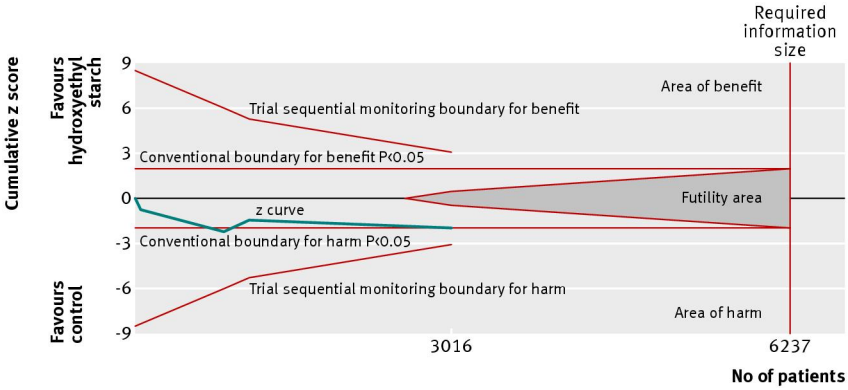


Fig 3 Trial sequential analysis of mortality in four trials with low risk of bias. A diversity adjusted information size of 6237 patients was calculated using α=0.05 (two sided), β=0.20 (power 80%), D^2=0%, an anticipated relative risk increase of 11%, and an event proportion of 30% in the control arm. The blue cumulative z curve was constructed using a random effects model

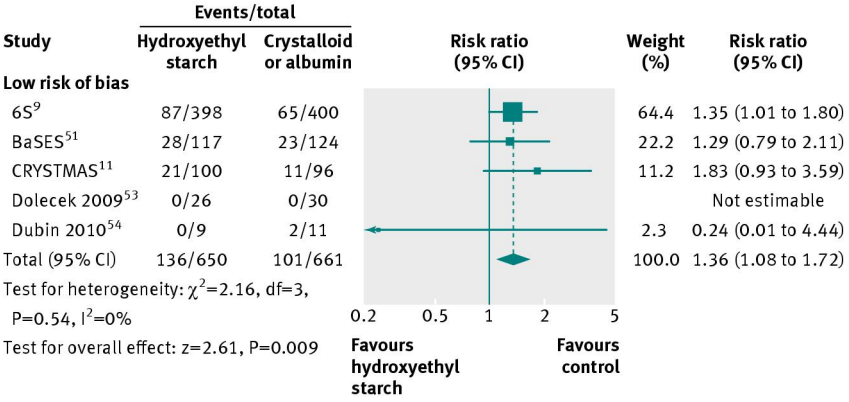


Fig 4 Forest plot of renal replacement therapy. Size of squares for risk ratio reflects weight of trial in pooled analyses. Horizontal bars represent 95% confidence intervals

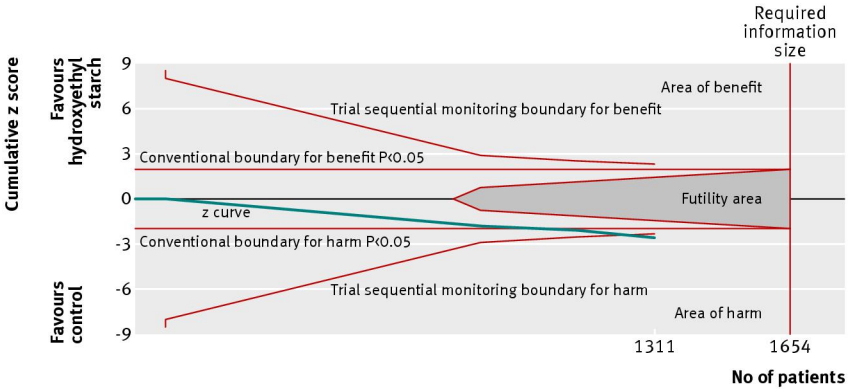


Fig 5 Trial sequential analysis of renal replacement therapy. A diversity adjusted information size of 1654 patients was calculated using α=0.05 (two sided), β=0.20 (power 80%), D^2=0%, an anticipated relative risk increase of 35% and an event proportion of 15% in the control arm. The blue cumulative z curve was constructed using a fixed effects model. Trials with no events were included in the analysis with an empirical continuity correction of 0.01

Supplementary appendix

This supplement contains the following items:

1. Search Strategy
2. Summary of included trials
3. Supplementary Figures

Search Strategy

Cochrane Central Register of Controlled Trials (CENTRAL) (Issue 8 of 12, 2012) in The Cochrane Library (705 hits in CENTRAL)

#1 MeSH descriptor Hetastarch explode all trees

#2 ((hydroxyet*yl and starch) or (HES and "130") or tetrastarch or hetastarch or Voluven or Volulyte or Tetraspan or Venofundin or Equihes or ISOHES or Restorvol or Venohes or Amidolite or Hesra)

#3 (#1 OR #2)

MEDLINE (Ovid SP)(1946 to September 2012)(2151 hits)

1. ((hydroxyet*yl and starch) or (HES and 130) or tetrastarch or hetastarch or Voluven or Volulyte or Tetraspan or Venofundin or Equihes or ISOHES or Restorvol or Venohes or Amidolite or Hesra).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

2. exp Hetastarch/

3. 1 or 2

EMBASE (Ovid SP)(1974 to September 2012)(3758 hits)

1. exp HETASTARCH/

2. ((hydroxyet*yl and starch) or (HES and 130) or tetrastarch or hetastarch or Voluven or Volulyte or Tetraspan or Venofundin or Equihes or ISOHES or Restorvol or Venohes or Amidolite or Hesra).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer]

3. 1 or 2

4. limit 3 to human

Science Citation Index Expanded (http://apps.webofknowledge.com)(1900 to September 2012)(3259 hits)

1 3,259 TS=((hydroxyet*yl and starch) or (HES and "130") or tetrastarch or hetastarch or Voluven or Volulyte or Tetraspan or Venofundin or Equihes or ISOHES or Restorvol or Venohes or Amidolite or Hesra)

BIOSIS Previews (http://apps.webofknowledge.com)(1969 to September 2012)(1395 hits)

3 1,395 #2 OR #1

2 598 TI=((hydroxyet*yl and starch) or (HES and "130") or tetrastarch or hetastarch or Voluven or Volulyte or Tetraspan or Venofundin or Equihes or ISOHES or Restorvol or Venohes or Amidolite or Hesra) AND Taxa Notes=(Humans)

1 1,395 TS=((hydroxyet*yl and starch) or (HES and "130") or tetrastarch or hetastarch or Voluven or Volulyte or Tetraspan or Venofundin or Equihes or ISOHES or Restorvol or Venohes or Amidolite or Hesra) AND Taxa Notes=(Humans)

CINAHL (EBSCO host)(1981 to November 2011)(279 hits)

S3 S1 or S2

S2 TX ((hydroxyet*yl and starch) or (HES and 130) or tetrastarch or hetastarch or Voluven or Volulyte or Tetraspan or Venofundin or Equihes or ISOHES or Restorvol or Venohes or Amidolite or Hesra)

S1 MM hydroxyethyl starch

6S-trial

Methods	• **Design:** RCT • **Setting:** Multicenter • **Blinding:** Yes

Participants	• **Country:** Denmark, Norway, Finland, Iceland
	• **Inclusion criteria:** Adults, severe sepsis and need of fluid resuscitation
	• **Surgical / medical:** 29% surgical, 71% medical
	• **Number of patients:**
	  ○ Group 1: 398
	  ○ Group 2: 400
	• **Age (median, IQR):**
	  • Group 1 : 66 (56-75)
	  • Group 2: 67 (56-76)
	• **SAPS II-score (median, IQR):**
	  • Group 1 : 50 (40-60)
	  • Group 2: 51 (39-62)
	Exclusion criteria: <18 y of age, RRT, kidney or liver transplantation, burn injury >10% of body surface, intracranial bleeding, serum potassium >6 mmol per liter within 6 hr before screening, included in another ICU trial, withdrawal from active therapy, received >1000 ml of synthetic colloid,

Interventions	• **Indication:** Fluid resuscitation, judged by the clinican, no predefined targets • **Dosing:** Max. 33 ml/kg/day. • **Intervention period:** ICU-stay, max. 90 day **Group 1:** • 6% HES 130/0.4 in Ringer's Acetate (Tetraspan®) • Total volume: 3000 (IQR: 1507-5100) **Group 2:** • Ringer's Acetate • Total volume 3000 (IQR: 2000-5750)
Outcomes	1.1 Overall mortality 1.2 RRT at end of follow-up 2.1 RRT 2.2 Creatinine x 2 2.3 RBC transfusion 2.4 Volume of transfused RBC 2.5 Bleeding episodes 2.6 Blood loss 2.7 Severe Adverse Reactions

	Time frame: 90 days for mortality and RRT. ICU-stay for other outcomes.	
Notes		
Risk of bias		
Bias	**Authors judgement**	**Support for judgement**
Random Sequence Generation	Low	Computer-generated
Allocation Concealment	Low	Phone/web-based randomisation
Blinding	Low	Blinded
Incomplete Outcome Data - mortality	Low	No missingness
Incomplete Outcome Data – other outcomes	Low	Missingness adequately described
Selective Outcome reporting	Low	
Baseline Imbalance	Low	No imbalance
Bias due to vested financial interests	Low	Funded by the Danish Research Councils. B Braun Medical delivered trial fluids to the trial sites free of charge. The contract between B Braun Medical AG and the sponsor ensures publication of the trial results independently of B Braun Medical AG.
Academic bias	Low	No previous trials on HES

BaSES (unpublished)

Reference: http://clinicaltrials.gov/ct2/show/NCT00273728

Methods	• **Design:** RCT • **Setting:** 2 ICUs in one hospital • **Blinding:** Yes

Participants	• **Country:** Switzerland
	• **Inclusion criteria:** Adults, with sepsis. Hypotension, oliguria or altered mental state could replace SIRS-criteria
	• **Number of patients:**
	  ○ Group 1: 117
	  ○ Group 2: 124
	• **Age (median, IQR):**
	  • Group 1 : 67 (50-75)
	  • Group 2: 68 (60-75)
	• **SOFA-score (median, IQR):**
	  • Group 1 : 3 (1-6)
	  • Group 2: 3 (1-6)
	• **APACHE II-score (median, IQR):**
	  • Group 1 : 21 (14-27)
	  • Group 2: 22 (13-28)
	Exclusion criteria: Pregnancy, age > 18y, allergy against HES products, chronic or acute kidney injury with Crea > 350 µmol/l

Interventions	• **Indication:** Fluid resuscitation. • **Dosing:** Every litre of study fluid was followed by one litre of Ringer's lactate. • **Intervention period:** 5 days **Group 1:** • HES 130/0.4 in Saline (Voluven®) • Total volume (median, IQR): 3775ml (2018-6347) • Additional Ringer's lactate (median, IQR): 5354ml (3015-8933) **Group 2:** • Isotonic Saline • Total volume (median, IQR): 4125ml (2500-6730) • Additional Ringer's lactate (median, IQR): 5770ml (3244-9930)
Outcomes	1.1 Overall mortality 1.2 RRT at end of follow-up 2.1 RRT **Time frame:** 1 year

Notes		
Risk of bias		
Bias	**Authors judgement**	**Support for judgement**
Random Sequence Generation	Low	Computer-generated
Allocation Concealment	Low	
Blinding	Low	Blinded
Incomplete Outcome Data - mortality	Low	No missingness
Incomplete Outcome Data – other outcomes	-	No data on other outcomes
Selective Outcome reporting	Low	Renal data has been registered and awaits publication.
Baseline Imbalance	Low	
Bias due to vested financial interests	Low	Fresenius Kabi delivered study fluids for free and paid for the packaging and blinding process. A signed contract between Fresenius and sponsor states that sponsor is free to publish all data without influence from Fresenius.
Academic Bias	Low	No previous studies on HES 130/0.4.

CHEST

Methods	• **Design:** RCT • **Setting:** Multicenter • **Blinding:** Yes	

Participants	• **Country:** Australia, New Zealand
	• **Inclusion criteria:** Adults patients in the ICU, need of fluid resuscitation. Predefined subgroup of patients with sepsis (n=1937)
	• **Number of patients:**
	  ○ Group 1: 979 (total: 3500)
	  ○ Group 2: 958 (total: 3500)
	• **Age, all patients (mean ± SD):**
	  • Group 1: 63 ± 17
	  • Group 2: 63 ± 17
	• **APACHE II-score, all patients (median, IQR):**
	  • Group 1 : 17 (12-22)
	  • Group 2: 17 (12-23)
	Exclusion criteria: <18 y of age, known allergy to starch, intracranial hemorrhage, RRT ongoing or imminent, creatinine > 350µmol/l, women aged 18-49 y unless negative pregnancy test, received >1000 ml starch already, cardiac surgical patients, burns, liver transplantation, imminent or inevitable death, underlying disease with a life expectancy of < 90 d, already received resuscitation fluid in the ICU, therapy limitations.	

Interventions	• **Indication:** Fluid resuscitation. • **Dosing:** 50 ml/kg/day • **Intervention period:** ICU-stay, max. 90 days **Group 1:** • HES 130/0.4 in Saline (Voluven®) • Daily volume in the first 4 days, all patients (mean ± SD): 526 ± 425 **Group 2:** • Isotonic Saline • Daily volume in the first 4 days, all patients (mean ± SD): 616 ± 488	
Outcomes	1.1 Overall mortality **Time frame:** 90 days	
Notes	Renal data of the sepsis subgroup are still unpublished.	
Risk of bias		
Bias	**Authors judgement**	**Support for judgement**
Random Sequence Generation	Low	Computer-generated

Allocation Concealment	Low	
Blinding	Low	Blinded
Incomplete Outcome Data - mortality	Low	Missingness < 1%
Incomplete Outcome Data – other outcomes	-	No data on other outcomes
Selective Outcome reporting	Low	Renal data has been registered and awaits publication.
Baseline Imbalance	Low	
Bias due to vested financial interests	Low	Fresenius Kabi supplied the study fluids and distributed them to participating sites. The trial was partly financed by an unrestricted grant from Fresenius Kabi. However, Fresenius Kabi had no input into the design, conduct, data collection, statistical analysis or writing of the manuscript.
Academic Bias	Low	No previous studies on HES 130/0.4.

CRYSTMAS

Other references:

Data published on clinicaltrials.gov

http://clinicaltrials.gov/ct2/show/NCT00464204

FDA Package insert (May 2, 2012)
http://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/NewDrugApplicationsNDAs/ucm082785.htm

Methods	• **Design:** RCT • **Setting:** Multicenter • **Blinding:** Yes

Participants	• **Country:** France, Germany
	• **Inclusion criteria:** Adults, severe sepsis and need of fluid resuscitation
	• **Number of patients:**
	  ○ Group 1: 100
	  ○ Group 2: 96
	• **Age (mean ± SD):**
	  • Group 1 : 65.8 ± 15.4
	  • Group 2: 65.9 ± 14.7
	Exclusion criteria: creatinine > 300 µM, chronic renal failure, anuria > 4 hours, RRT

Interventions	• **Indication:** Fluid resuscitation • **Dosing:** Max. 50 ml/kg on day 1. Max. 25 ml/kg days 2 to 4. • **Intervention period:** 4 days **Group 1:** • HES 130/0.4 in saline (Voluven®) • Total volume (mean ± SD): 2615 ± 1499 **Group 2:** • Isotonic Saline • Total volume (mean ± SD): 2788 ± 1799
Outcomes	1.1 Overall mortality 2.1 RRT 2.2 Creatinine x 2 2.3 RBC transfusion 2.4 Volume of transfused RBC 2.6 Blood loss 2.7 Severe Adverse Events **Time frame:** Mortality, RRT and Severe Adverse Events: 90 days. Other endpoints: ICU-stay

Notes	Data on dialysis were not reported in the publication, but were retrieved from the FDA package insert for Voluven. Data on blood loss and haemorrhage were delivered by Fresenius Kabi: **Haemorrhage** Voluven: 9 of 100 Saline: 10 of 96 Observation period: 8 days **Blood loss** Voluven: 468 ± 1454 in 100 patients Saline: 456 ± 1398 in 96 patients Observation period: 4 days	
Risk of bias		
Bias	**Authors judgement**	**Support for judgement**
Random Sequence Generation	Low	Reply from author
Allocation Concealment	Low	Reply from author
Blinding	Low	Reply from author
Incomplete Outcome Data – mortality	Low	All patients were analysed
Incomplete Outcome Data – other outcomes	Low	All patients were analysed
Selective Outcome reporting	Low	All outcomes of relevance to this review are reported in either the paper or in the FDA package insert or retrieved from the authors. However, one nutrition outcome was changed from primary to secondary on clinicaltrials.gov after end of trial.
Baseline Imbalance	Low	
Bias due to vested financial interests	High	Conducted by the manufacturer of Voluven®, Fresenius Kabi AG
Academic Bias	High	Primary investigator has previously made smaller studies of HES and published reviews of HES focusing on effectiveness of HES 130/0.4 and the few

		adverse effects compared with other starches.

Dolecek 2009

Methods	• **Design:** RCT • **Setting:** Single center • **Blinding:** No

Participants	• **Country:** Czech Republic
	• **Inclusion criteria:** Adults with severe sepsis on the ventilator. Extra vascular lung water index > 7 ml/kg as measured by PiCCO.
	• **Surgical / medical:** both
	• **Number of patients:**
	  ○ Group 1: 26
	  ○ Group 2: 30
	• **Age (mean, range):**
	  • Group 1 : 43 (23-67)
	  • Group 2: 47 (19-81)
	• **SOFA-score (mean ± SD):**
	  • Group 1 : 8.8 ± 3.0
	  • Group 2: 8.0 ± 2.0
	
	Exclusion criteria: severe coagulopathy, pregnancy, cardiac failure, AKF, limitations for PiCCO - aortic aneurism, severe aortal regurgitation, dysrythmia

Interventions	• **Indication:** Fixed dose • **Dosing:** see below. • **Intervention period:** 3 days **Group 1:** • HES 130/0.4 in saline (Voluven®). • Total volume: 4 x 250 ml per day in 3 days **Group 2:** • Albumin 20% • Total volume: 2 x 100 ml per day in 3 days
Outcomes	1.1 Overall mortality 1.2 RRT at end of follow-up 2.1 RRT 2.2 Creatinine x 2 2.7 Severe Adverse Reactions **Time frame:** mortality: 28 days; RRT, creatinine and serious adverse events: 3 days
Notes	The authors now work in another hospital and have no longer access to source data on renal replacement therapy, bleeding or blood transfusions.
Risk of bias	

Bias	Authors judgement	Support for judgement
Random Sequence Generation	Low	Computer generated, mail from author
Allocation Concealment	Low	Sealed envelopes
Blinding	Low for mortality and creatinine / high for RRT if we get these data	Unblinded treatment, but study fluid was given in fixed doses.
Incomplete Outcome Data - mortality	Low	CONSORT diagram in paper
Incomplete Outcome Data – other outcomes	Low	
Selective Outcome reporting	Low	
Baseline Imbalance	Low	No imbalance
Bias due to vested financial interests	Low	None declared
Academic Bias	Low	

Dubin 2010

Methods	• **Design:** RCT • **Setting:** 2 centers • **Blinding:** No

Participants	• **Country:** Argentina
	• **Inclusion criteria:** Adults, sepsis and tissue hypoperfusion
	• **Surgical / medical:** both
	• **Number of patients:**
	  ○ Group 1: 12
	  ○ Group 2: 13
	• **Age (mean ± SD):**
	  • Group 1 : 62 ± 21
	  • Group 2: 65 ± 12
	• **SOFA-score (mean ± SD):**
	  • Group 1 : 8.1 ± 2.5
	  • Group 2: 8.9 ± 3.6
	
	Exclusion criteria: Not possible to perform lingual videomicroscopy, < 18y, pregnancy, stroke, acute coronary syndrome, hydrostatic pulmonary edema, status astmaticus, cardiac arrhythmias, contraindication for central venous catheterization, active gastrointestinal haemorrhage, seizures, drug intoxication, burns, trauma, need of imidiate surgery, terminal cancer, immunosuppression, no resuscitation order, delayed admission til ITA (more than 4 hours), previous resuscitation with > 1500 ml fluid

Interventions	• **Indication:** Fluid resuscitation • **Dosing:** NS • **Intervention period:** 24 hours **Group 1:** • HES 130/0.4 in saline (Voluven®) • Total volume (mean ± SD): 2610 ± 885 **Group 2:** • Isotonic saline • Total volume (mean ± SD): 6254 ± 2603
Outcomes	1.1 Overall mortality 1.2 RRT at 90 days 2.1 RRT 2.2 Creatinine x 2 2.3 RBC transfusion 2.4 Volume of transfused RBC

	2.7 Severe Adverse Reactions **Time frame:** Mortality 28 days. Other outcomes: 24 hours.	
Notes		
Risk of bias		
Bias	**Authors judgement**	**Support for judgement**
Random Sequence Generation	Low	Computer-generated
Allocation Concealment	Low	Sealed envelopes
Blinding	High	No blinding
Incomplete Outcome Data - mortality	Low	Consort diagram in paper
Incomplete Outcome Data – other outcomes	Low	
Selective Outcome reporting	Low	
Baseline Imbalance	Low	No imbalance
Bias due to vested financial interests	Low	None declared
Academic Bias	Low	

Lv 2012

Methods	• **Design:** RCT • **Setting:** Single center • **Blinding:** Unclear	

Participants	• **Country:** China
	• **Inclusion criteria:** Septic shock, > 18 years old, > 30 ml/kg fluid received in 24 hours,
	• **Number of patients:**
	  ○ Group 1: 22
	  ○ Group 2: 20
	• **Age** (mean ± SD)**:**
	  • Group 1 : 66 ± 15
	  • Group 2: 65 ± 14
	Exclusion criteria: Blood products received in the last 24h, history of bleeding or coagulation disorder, receiving drug with potential impact on coagulation.	

Interventions	• **Indication:** Fluid resuscitation • **Dosing:** Max. dose not defined. • **Intervention period:** 24 hours **Group 1:** • 6% HES 130/0.4 (unknown carrier solution) • Total volume (mean ± SD): 2.8 ± 0.6 **Group 2:** • Ringer's lactate • Total volume (mean ± SD): 3.5 ± 0.7	
Outcomes	1.1 Overall mortality **Time frame:** Not specifically stated, but appears to be the entire ICU stay	
Notes		
Risk of bias		
Bias	**Authors judgement**	**Support for judgement**
Random Sequence Generation	Low	"Random table"
Allocation Concealment	High	Not described
Blinding (RRT, transfusion)	Unclear	Not mentioned
Incomplete Outcome Data - mortality	Unclear	Not stated that there were no dropouts
Incomplete Outcome Data – other outcomes	-	No data
Selective Outcome reporting	Low	Mortality and ICU length of stay are accounted for. No detailed information on kidney function.
Baseline Imbalance	Low	

Bias due to vested financial interests	Unclear	
Academic Bias	Unclear	

Palumbo 2006

Methods	• **Design:** RCT • **Setting:** Single center • **Blinding:** Unclear	

Participants	• **Country:** Italy
	• **Inclusion criteria:** Severe sepis
	• **Surgical / medical:** 7 medical, 13 surgical
	• **Number of patients:**
	  • Group 1: 10
	  • Group 2: 10
	• **Age (mean ± SD):**
	  • All patients: 59.6 ± 12.6
	• **APACHE (mean ± SD):**
	  • All patients: 19.0 ± 3.6
	
	Exclusion criteria: < 21 years, renal dysfunction (serum creatinin > 2.0 mg/dl, blood nitrogen > 150 mg/dl, urine output < 20 ml/h in spite of diuretic therapy with furosemide), severe liver failure, DIC syndrome, considered to be in terminal state	

Interventions	• **Indication:** To maintain capillary wedge pressure • Dosing: NS • **Intervention period:** NS, but probably 5 days **Group 1:** • HES 130/0.4 in saline (Voluven®) • Total volume: NS **Group 2:** • Albumin: 20% • Total volume: NS	
Outcomes	No data included in the review	
Notes		
Risk of bias		
Bias	**Authors judgement**	**Support for judgement**
Random Sequence Generation	Unclear	Not reported
Allocation Concealment	High	Not reported, “assigned in a randomized sequence”
Blinding	Unclear	Not stated
Incomplete Outcome Data - mortality	Low	All patients followed-up
Incomplete Outcome Data – other outcomes	-	No other outcomes reported.
Selective Outcome reporting	High	Mortality is reported for

		each single patient without group assignment. Kidney function not reported.
Baseline Imbalance	Low	“groups were homogeneous for age, sex and pathology”
Bias due to vested financial interests	Unclear	Not stated
Academic Bias	Low	

Zhu 2011

Methods	• **Design:** RCT • **Setting:** Single center • **Blinding:** No	

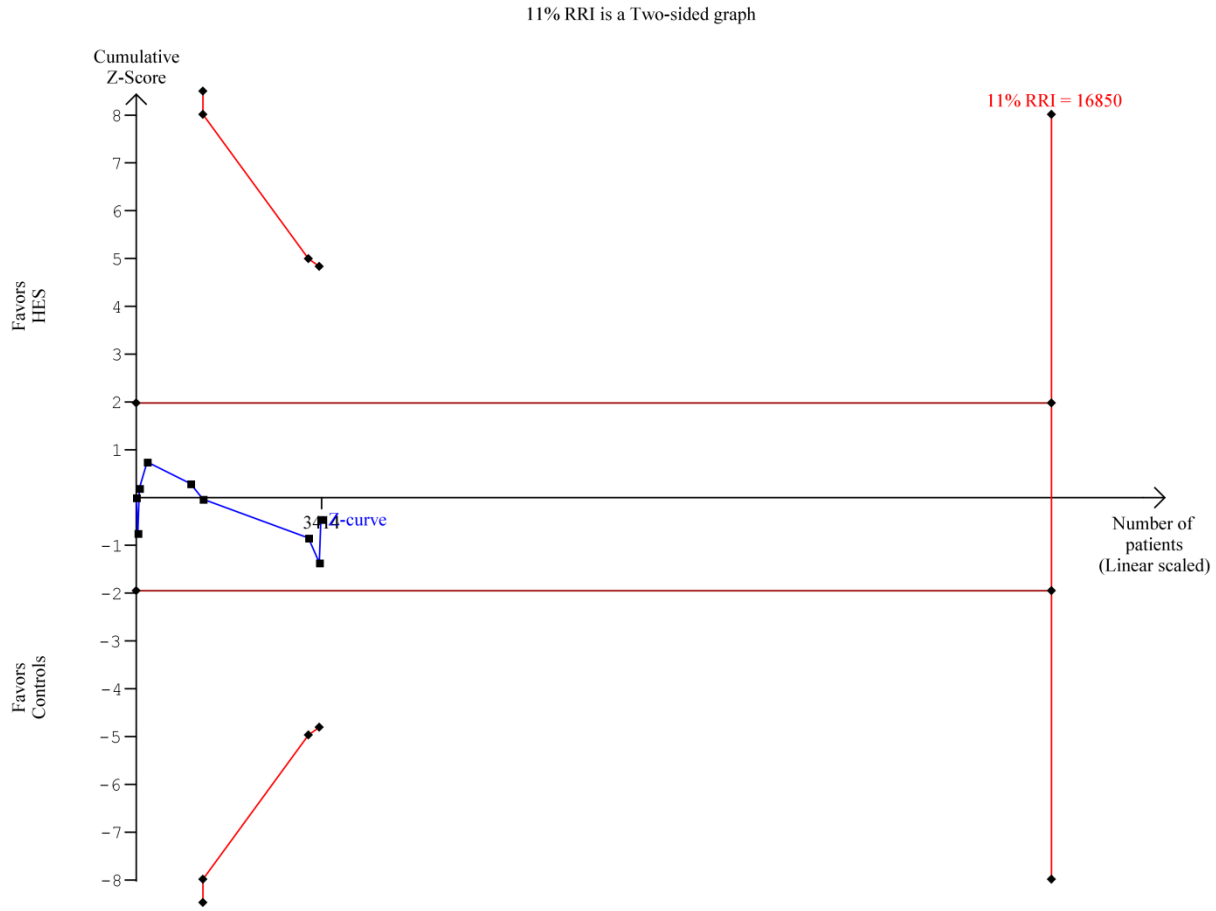
Participants	• **Country:** China
	• **Inclusion criteria:** Severe sepsis, adults
	• **Surgical / Medical:** all medical
	• **Number of patients:**
	  ○ Group 1: 45
	  ○ Group 2: 45
	  ○ Group 3: 45
	• **Age (mean ± SD):**
	  • Group 1 : 59.4 ± 8.8
	  • Group 2: 59.9 ± 9.4
	  • Group 3: 59.8 ± 9.3
	• **APACHE II-score (mean ± SD):**
	  • Group 1 : 17.3 ± 1.8
	  • Group 2: 17.0 ± 1.6
	  • Group 3: 17.2 ± 1.7
	Exclusion criteria: < 21 years, creatinine > 450 µmol/l, severe liver dysfunction, DIC or end-stage disease.	

Interventions	• **Indication:** Fluid resuscitation • **Dosing:** NS • **Intervention period:** Until urine output > 1 ml/kg for 1 hour OR 24 hours **Group 1:** • HES 130/0.4 in hypertonic saline • Total volume: 5476 ± 209 **Group 2:** • HES 130/0.4 in saline • Total volume: 6383 ± 287 **Group 3:** • Ringer's lactate • Total volume: 7439 ± 230	
Outcomes	1.1 Overall mortality **Time frame:** 24 hours.	
Notes		
Risk of bias		
Bias	**Authors judgement**	**Support for judgement**
Random Sequence Generation	Unclear	Not stated
Allocation Concealment	High	Not stated
Blinding	High	
Incomplete Outcome Data - mortality	Unclear	Not stated that there

		were no dropouts
Incomplete Outcome Data – other outcomes	-	No data
Selective Outcome reporting	Low	Mortality and organ failures are accounted for. No detailed information on kidney function.
Baseline Imbalance	Low	
Bias due to vested financial interests	Low	Financial support from Pharmaceutical Health Research Program of Hubei province
Academic Bias	Unclear	

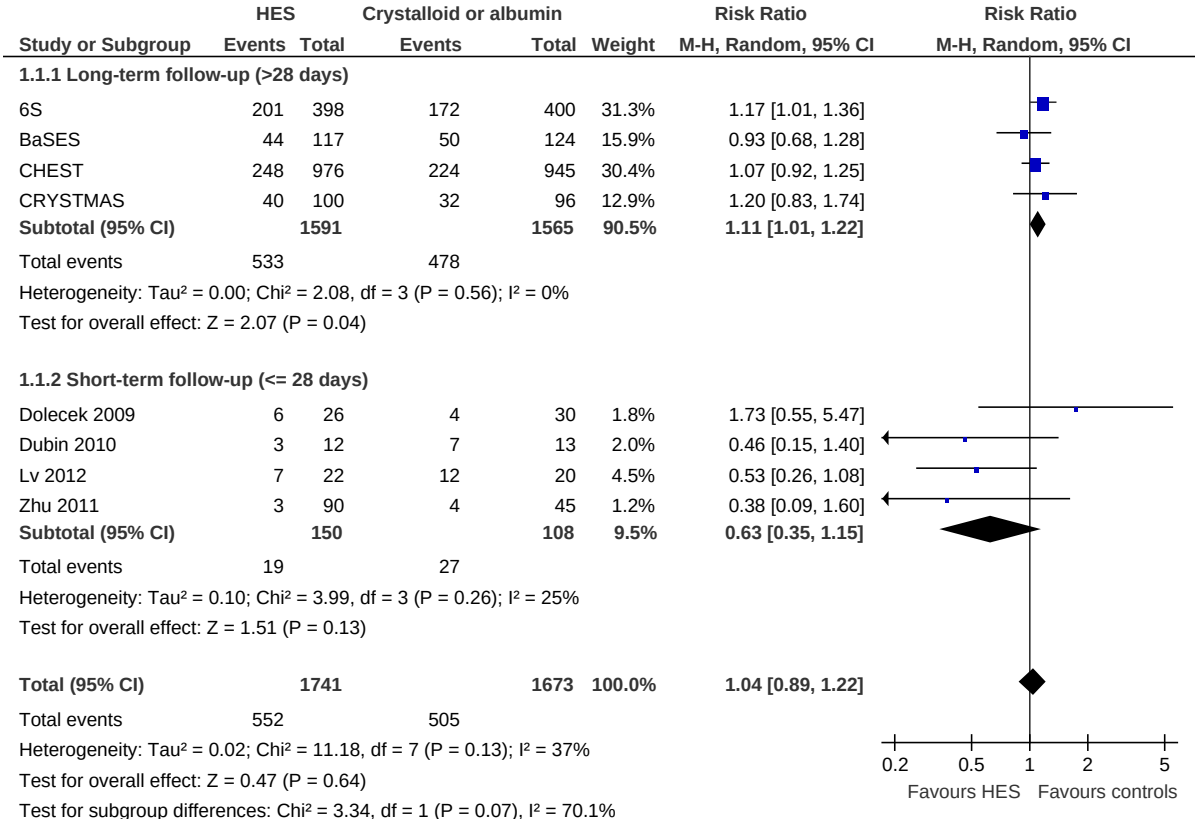
Supplementary figures

Trial Sequential Analysis of mortality in all trials

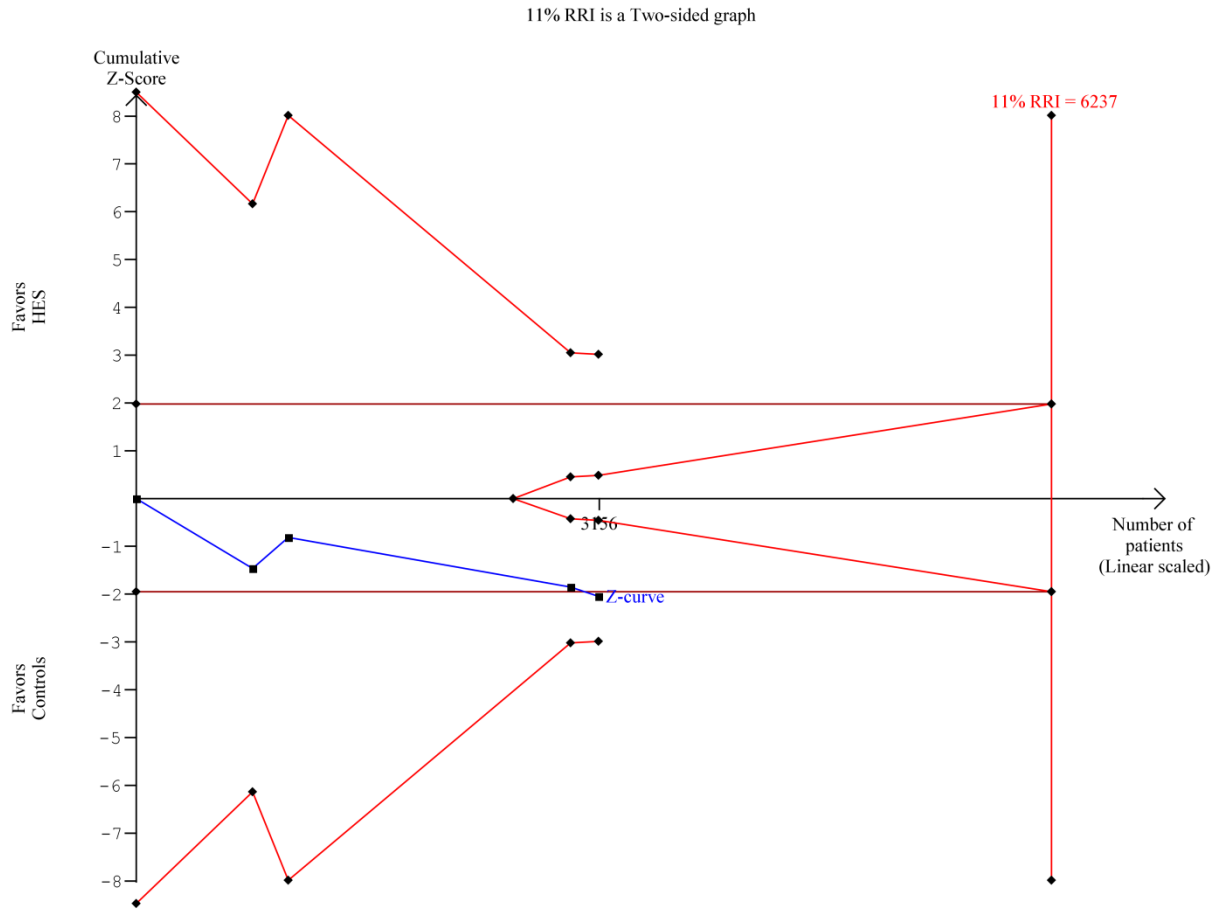


We calculated a diversity-adjusted information size of 16850 patients using α = 0.05 (two-sided), β = 0.20 (power = 80%), D^2= 63%, an anticipated relative risk increase of 11% and an event proportion of 30% in the control arm. The cumulative z-curve (blue) was constructed using a random-effects model. Only approximately 20% of the required information size was reached. The TSA adjusted confidence interval was 0.70 to 1.54.

Forest plot of trials with long-term (> 28 days) follow-up vs. trials with short-term follow-up (≤ 28 days)

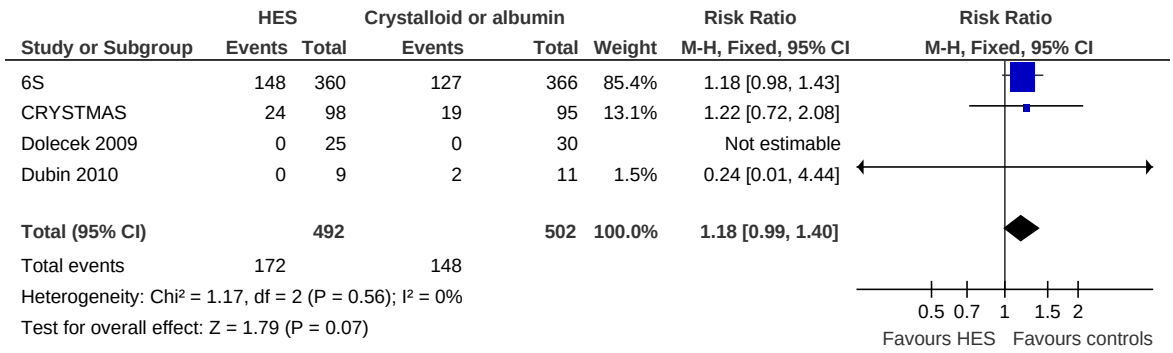


Trial Sequential Analysis of mortality in trials with follow-up for more than 28 days



We calculated a diversity-adjusted information size of 6237 patients using α = 0.05 (two-sided), β = 0.20 (power = 80%), D^2= 0%, an anticipated relative risk increase of 11% and an event proportion of 30% in the control arm. The cumulative z-curve (blue) was constructed using a random-effects model. The required information size was not reached and the z-curve crossed the conventional boundary, but not the trial sequential monitoring boundary for harm. The TSA adjusted confidence interval was 0.95 to 1.29. The z-curve needs to pass through the futility area to reach the area of benefit leaving little chance that HES will turn out to reduce the relative risk of death with 11% if further trials are conducted in patients with sepsis.

Forest plot of acute kidney injury (two-fold increase in creatinine)

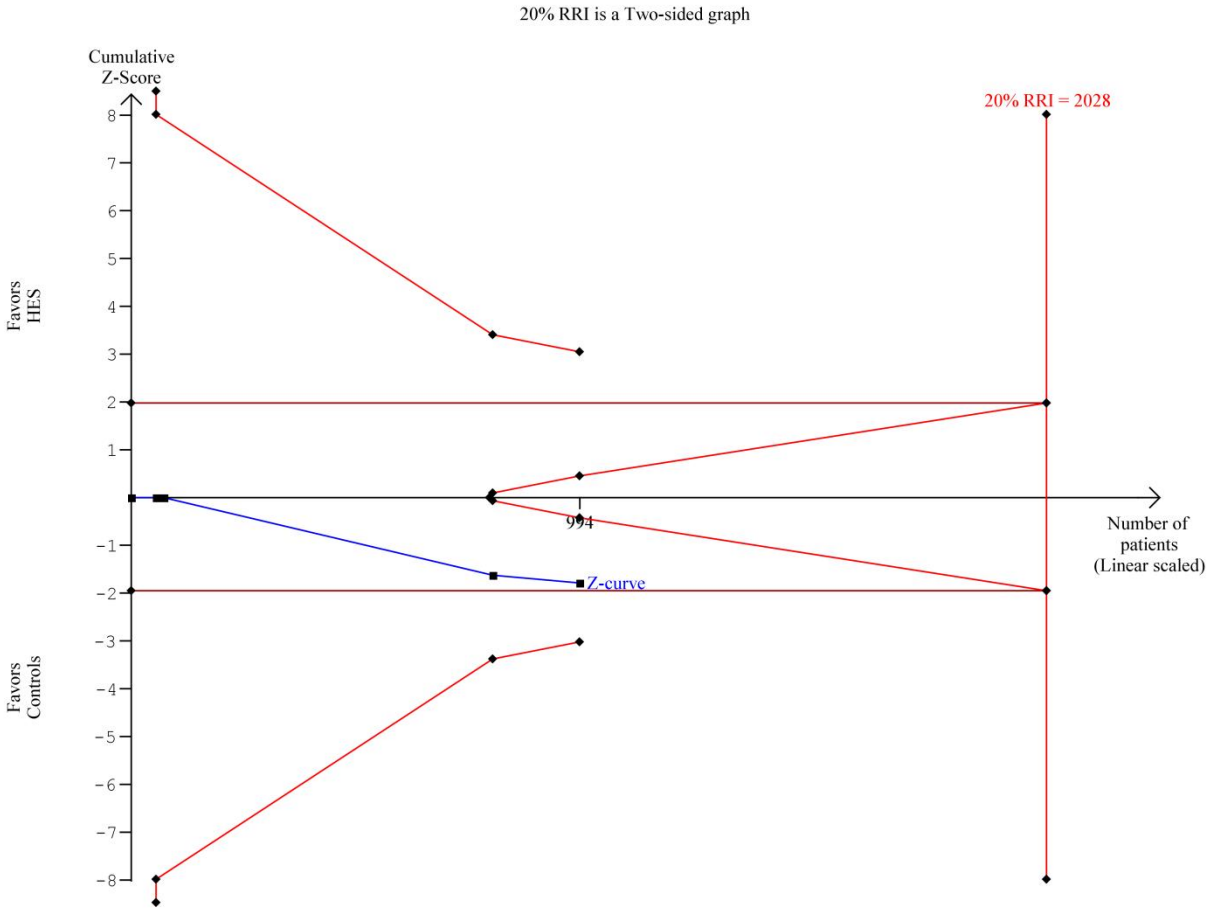


Study or Subgroup	HES Events	HES Total	Crystalloid or albumin Events	Crystalloid or albumin Total	Weight	Risk Ratio M-H, Fixed, 95% CI
6S	148	360	127	366	85.4%	1.18 [0.98, 1.43]
CRYSTMAS	24	98	19	95	13.1%	1.22 [0.72, 2.08]
Dolecek 2009	0	25	0	30		Not estimable
Dubin 2010	0	9	2	11	1.5%	0.24 [0.01, 4.44]
Total (95% CI)		**492**		**502**	**100.0%**	**1.18 [0.99, 1.40]**
Total events	172		148			

Heterogeneity: Chi² = 1.17, df = 2 (P = 0.56); I² = 0%

Test for overall effect: Z = 1.79 (P = 0.07)

Trial sequential analysis of acute kidney injury (two-fold increase in creatinine)



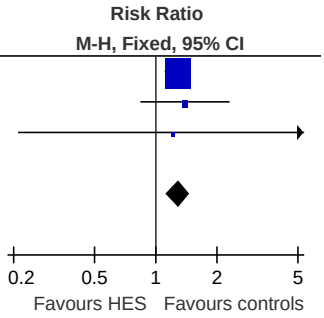
We calculated a diversity-adjusted information size of 2028 patients using α = 0.05 (two-sided), β = 0.20 (power = 80%), D^2= 0%, an anticipated relative risk increase of 20% and an proportion rate of 29% in the control arm. The cumulative z-curve (blue) was constructed using a fixed-effects model. Trials with no events were included in the analysis with an empirical continuity correction of 0.01. The required information size was not reached, and the z-curve did not cross the trial sequential monitoring boundary for harm. The TSA adjusted confidence interval was 0.90 to 1.54.

Forest plot of transfusion with red blood cells

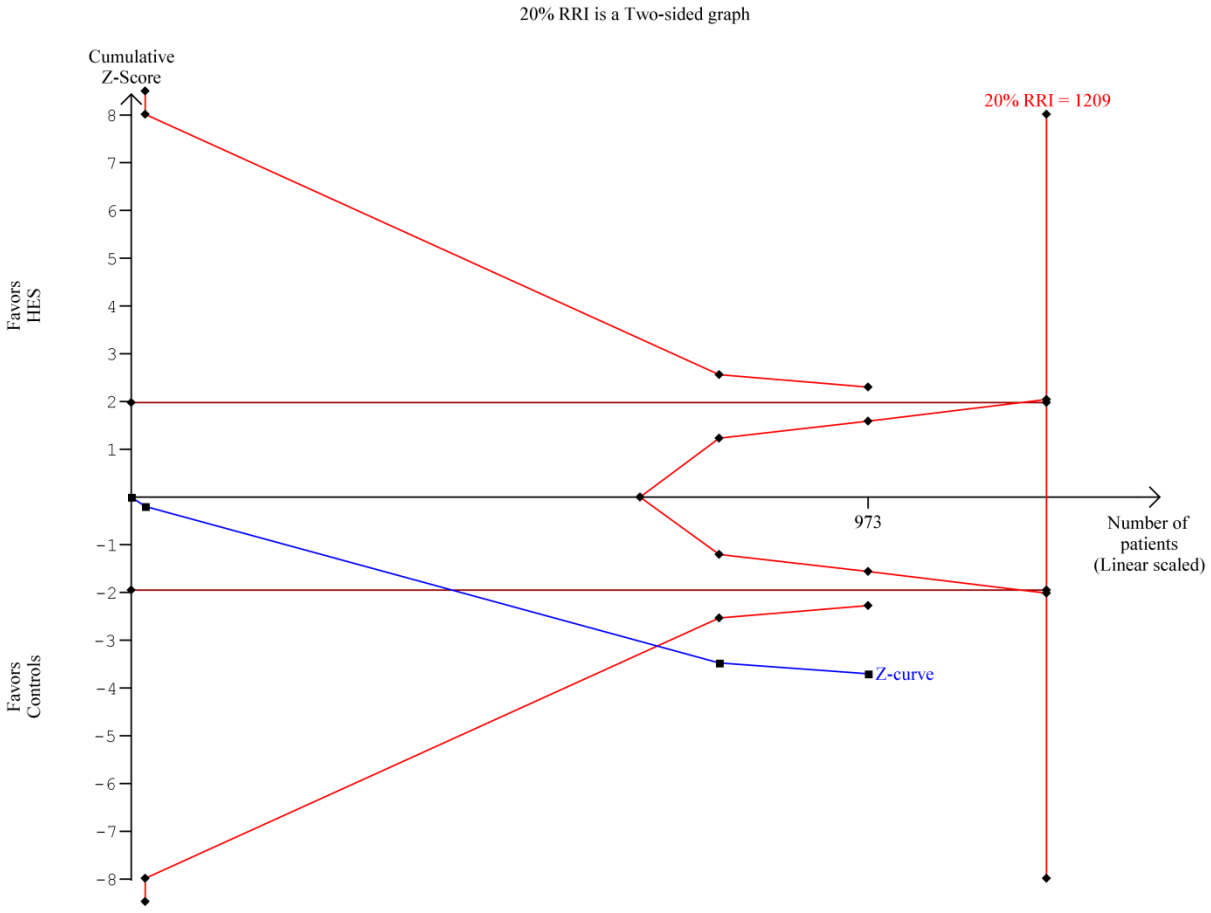
Study or Subgroup	HES Events	HES Total	Crystalloid or albumin Events	Crystalloid or albumin Total	Weight	Risk Ratio M-H, Fixed, 95% CI
6S	220	377	173	380	88.6%	1.28 [1.12, 1.47]
CRYSTMAS	29	100	20	96	10.5%	1.39 [0.85, 2.29]
Dubin 2010	2	9	2	11	0.9%	1.22 [0.21, 7.04]
Total (95% CI)		**486**		**487**	**100.0%**	**1.29 [1.13, 1.48]**
Total events	251		195			

Heterogeneity: Chi² = 0.10, df = 2 (P = 0.95); I² = 0%

Test for overall effect: Z = 3.73 (P = 0.0002)



Trial sequential analysis of transfusion with red blood cells



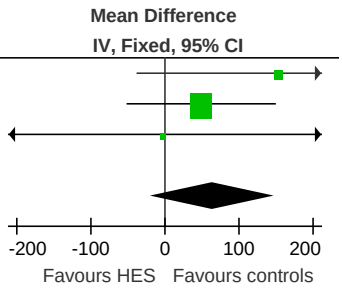
We calculated a diversity-adjusted information size of 1209 patients using α = 0.05 (two-sided), β = 0.20 (power = 80%), D^2= 0%, an anticipated relative risk increase of 20% and an event proportion of 40% in the control arm. The cumulative z-curve (blue) was constructed using a fixed-effects model. The required information size was not reached, but the z-curve crossed the trial sequential monitoring boundary for harm and the TSA adjusted confidence interval was 1.10 to 1.51 providing firm evidence of increased use of red blood cell transfusion in patients treated with HES vs. crystalloid or albumin.

Forest plot of volume of red blood cell transfusion

Study or Subgroup	HES Mean	HES SD	HES Total	Crystalloid or albumin Mean	Crystalloid or albumin SD	Crystalloid or albumin Total	Weight	Mean Difference IV, Fixed, 95% CI
6S	810	1,442	377	657	1,218	380	19.5%	153.00 [-37.22, 343.22]
CRYSTMAS	214	358	100	165	354	96	71.2%	49.00 [-50.69, 148.69]
Dubin 2010	133	265	9	136	364	11	9.3%	-3.00 [-279.12, 273.12]
Total (95% CI)			**486**			**487**	**100.0%**	**64.51 [-19.60, 148.61]**

Heterogeneity: Chi² = 1.15, df = 2 (P = 0.56); I² = 0%

Test for overall effect: Z = 1.50 (P = 0.13)

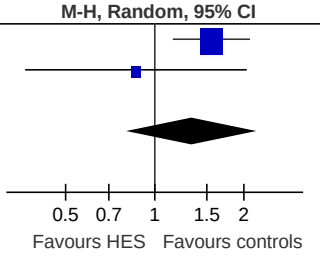


Forest plot of bleeding episodes

Study or Subgroup	HES Events	HES Total	Crystalloid or albumin Events	Crystalloid or albumin Total	Weight	Risk Ratio M-H, Random, 95% CI
6S	93	398	60	399	74.4%	1.55 [1.16, 2.08]
CRYSTMAS	9	100	10	96	25.6%	0.86 [0.37, 2.03]
Total (95% CI)		**498**		**495**	**100.0%**	**1.34 [0.81, 2.21]**
Total events	102		70			

Heterogeneity: Tau² = 0.07; Chi² = 1.62, df = 1 (P = 0.20); I² = 38%

Test for overall effect: Z = 1.13 (P = 0.26)

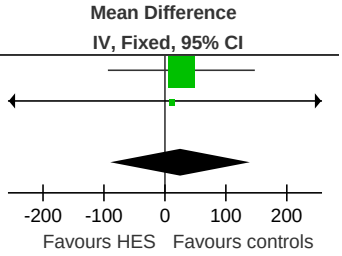


Forest plot of blood loss

Study or Subgroup	HES Mean	HES SD	HES Total	Crystalloid or albumin Mean	Crystalloid or albumin SD	Crystalloid or albumin Total	Weight	Mean Difference IV, Fixed, 95% CI
6S	192	799	398	165	915	399	91.8%	27.00 [-92.26, 146.26]
CRYSTMAS	468	1,454	100	456	1,398	96	8.2%	12.00 [-387.27, 411.27]
Total (95% CI)			**498**			**495**	**100.0%**	**25.77 [-88.50, 140.04]**

Heterogeneity: Chi² = 0.00, df = 1 (P = 0.94); I² = 0%

Test for overall effect: Z = 0.44 (P = 0.66)

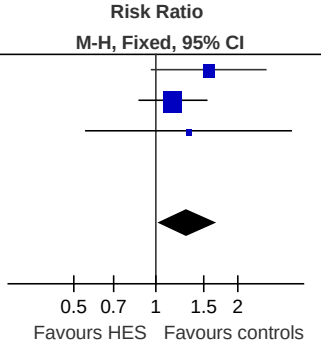


Forest plot of severe adverse events

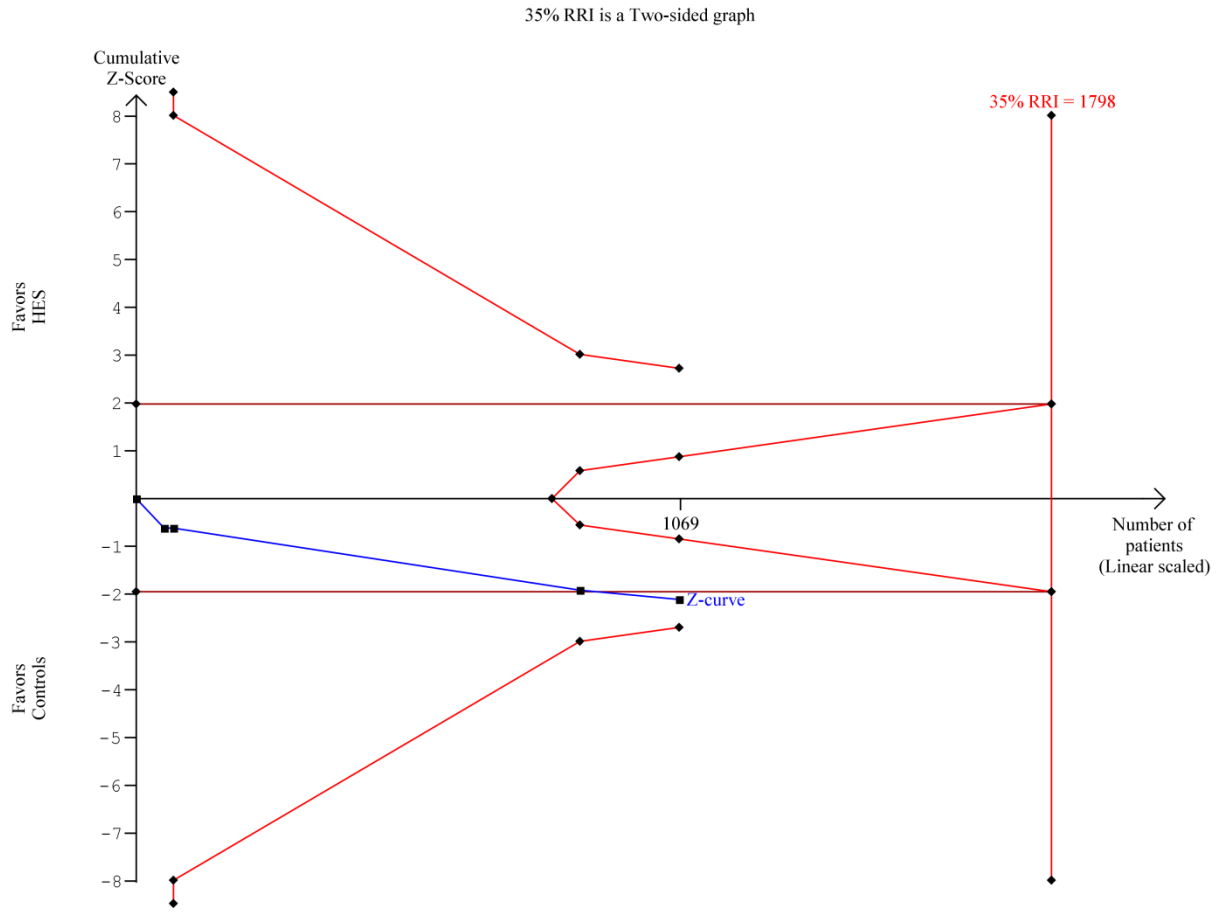
Study or Subgroup	HES Events	HES Total	Crystalloid or albumin Events	Crystalloid or albumin Total	Weight	Risk Ratio M-H, Fixed, 95% CI
6S	39	398	25	399	32.7%	1.56 [0.97, 2.53]
CRYSTMAS	53	100	44	96	58.8%	1.16 [0.87, 1.54]
Dolecek 2009	8	26	7	30	8.5%	1.32 [0.55, 3.14]
Dubin 2010	0	9	0	11		Not estimable
Total (95% CI)		**533**		**536**	**100.0%**	**1.30 [1.02, 1.67]**
Total events	100		76			

Heterogeneity: Chi² = 1.23, df = 2 (P = 0.54); I² = 0%

Test for overall effect: Z = 2.11 (P = 0.03)



Trial sequential analysis of severe adverse reactions



We calculated a diversity-adjusted information size of 1798 patients using α = 0.05 (two-sided), β = 0.20 (power = 80%), D^2= 0%, an anticipated relative risk increase of 35% and an event proportion of 14% in the control arm. The cumulative z-curve (blue) was constructed using a fixed-effects model. One trials with no events were included in the analysis with an empirical continuity correction of 0.01.The required information size was not reached and the z-curve crossed the conventional boundary, but not the trial sequential monitoring boundary for harm. The TSA adjusted confidence interval was 0.93 to 1.83.

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UNIVERSITY OF COPENHAGEN

DECLARATION OF CO-AUTHORSHIP

This form must be filled in on the screen, printed, signed and sent to the Graduate School
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Information on PhD student:	
Name of PhD student	Nicolai Rosenkrantz Segelcke Haase
E-mail	nicolai.rosenkrantz.segelcke.haase@regionh.dk
Date of birth	24-sep-1980
Work place	Intensiv Terapiklinik 4131, Rigshospitalet
Principal supervisor	Professor Anders Perner

Title of PhD thesis:

Hydroxyethyl Starch in Sepsis

This declaration concerns the following article:

Hydroxyethyl starch 130/0.42 versus Ringer's acetate in severe sepsis

The PhD student's contribution to the article: *(please use the scale (A,B,C) below as benchmark*)*	(A,B,C)
1. Formulation/identification of the scientific problem that from theoretical questions need to be clarified. This includes a condensation of the problem to specific scientific questions that is judged to be answerable by experiments	B
2. Planning of the experiments and methodology design, including selection of methods and method development	B
3. Involvement in the experimental work	B
4. Presentation, interpretation and discussion in a journal article format of obtained data	B

**Benchmark scale of the PhD student's contribution to the article*		
A. refers to:	*Has contributed to the co-operation*	*0-33 %*
B. refers to:	*Has contributed considerably to the co-operation*	*34-66 %*
C. refers to:	*Has predominantly executed the work Independently*	*67-100 %*

Signature of the co-authors:

Date:	Name:	Title:	Signature:
10-06-2013	Jørn Wetterslev	Chief Physiscian, MD, PhD	

Signature of the PhD student and the principal supervisor:

Date: 10/6-13 NHaase PhD student:	Date: 21/6/13 Principal supervisor:

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Principal supervisor	Professor Anders Perner

Title of PhD thesis:
Hydroxyethyl Starch in Sepsis

This declaration concerns the following article:
Bleeding and Risk of Death with Hydroxyethyl Starch in Severe Sepsis

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Signature of the co-authors:			
Date:	Name:	Title:	Signature:
17/7-2013	Per Winkel	MD. dr. med	Per Winkel.

Signature of the PhD student and the principal supervisor:	
Date: 21/6-13 PhD student: NHaase	Date: 21/6/13 Principal supervisor:

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PhD student: NHaase	Principal supervisor:

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Signature of the co-authors:			
Date:	Name:	Title:	Signature:
11/6-13	Louise Inkeri Hennings	physician	

Signature of the PhD student and the principal supervisor:	
Date: 11/6-13 PhD student: NHaase	Date: 21/6/13 Principal supervisor:

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Signature of the co-authors:

Date:	Name:	Title:	Signature:
10.06.2013	Martin Siegemund	PD Dr. med.	

Signature of the PhD student and the principal supervisor:

Date: 11/6-13	Date: 24/6/13
PhD student: N Haase	Principal supervisor:

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DECLARATION OF CO-AUTHORSHIP

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Information on PhD student:	
Name of PhD student	Nicolai Rosenkrantz Segelcke Haase
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Date of birth	24-sep-1980
Work place	Intensiv Terapiklinik 4131, Rigshospitalet
Principal supervisor	Professor Anders Perner

Title of PhD thesis:
Hydroxyethyl Starch in Sepsis

This declaration concerns the following article:
Hydroxyethyl starch 130/0.38-0.45 versus crystalloid or albumin in patients with sepsis: systematic review with meta-analysis and trial sequential analysis

The PhD student's contribution to the article: *(please use the scale (A,B,C) below as benchmark*)*	(A,B,C)
1. Formulation/identification of the scientific problem that from theoretical questions need to be clarified. This includes a condensation of the problem to specific scientific questions that is judged to be answerable by experiments	C
2. Planning of the experiments and methodology design, including selection of methods and method development	C
3. Involvement in the experimental work	C
4. Presentation, interpretation and discussion in a journal article format of obtained data	C

**Benchmark scale of the PhD student's contribution to the article*		
A. refers to:	*Has contributed to the co-operation*	*0-33 %*
B. refers to:	*Has contributed considerably to the co-operation*	*34-66 %*
C. refers to:	*Has predominantly executed the work independently*	*67-100 %*

Signature of the co-authors:			
Date:	Name:	Title:	Signature:
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Signature of the PhD student and the principal supervisor:	
Date: 10/6-13 PhD student: NHaase	Date: 2/6/13 Principal supervisor:

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UNIVERSITY OF COPENHAGEN

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Signature of the co-authors:			
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Date: 14/6-13

PhD student: NHaase

Date: 2/6/13

Principal supervisor

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PhD student: NHaase	Principal supervisor: