Hydroxyethyl Starch In Sepsis

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ORIGINAL PAPERS

1. Perner A, Haase N, Guttormsen AB, et al. Hydroxyethyl starch 130/0.42 versus Ringer's acetate in severe sepsis. N Engl J Med 2012; 367: 124–34
2. 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 – Post-hoc analyses of a randomised clinical trial. Intensive Care Med 2013; 39: 2126-34
3. Haase N, Perner A, Hennings LI, et al. 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

1. INTRODUCTION

The new generation of hydroxyethyl starch (HES), tetrastarch, was launched in 1999 as a promising colloid providing efficient restoration of circulation without causing 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.

2. 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, but 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.
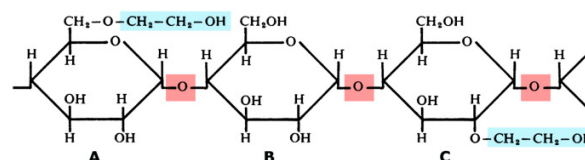

Figure 1
Simplified structure of HES. The figure shows a chain of glucose units (labelled A, B and C) with hydroxyethyl groups attached (blue). Two of three glucose units are hydroxyethylated. Hydroxyethyl is bound to C6 at glucose unit A and to C2 at glucose unit C. Amylase splits the chain of glucose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

HES is metabolized 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 metabolization 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].
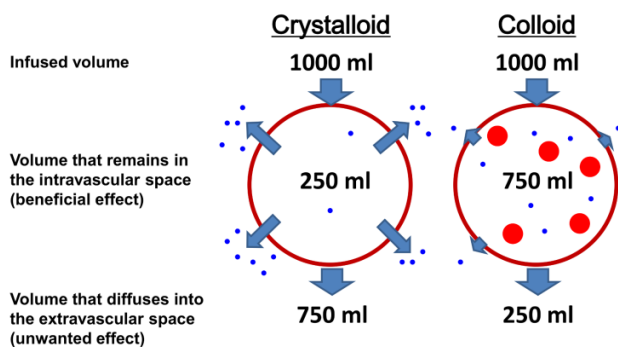

Figure 2
A simple physiological model of fluid compartments and membranes constituting the rationale for the use of colloids instead of crystalloids. The crystalloids consist of small molecules (blue), which diffuse across the endothelial barrier to the extracellular space and draw water with them. The 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.

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].

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.

3. 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 controlled trial. Secondarily, we aimed at comparing our results with those of similar trials in a systematic review.

4. 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 [27]. Paper II contains post-hoc analyses of the relationships among type of trial fluid, haemostatic variables, bleeding and mortality [28].

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 [29].

5. 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 content 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 [30, 31].

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 >1000 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 blood) 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).
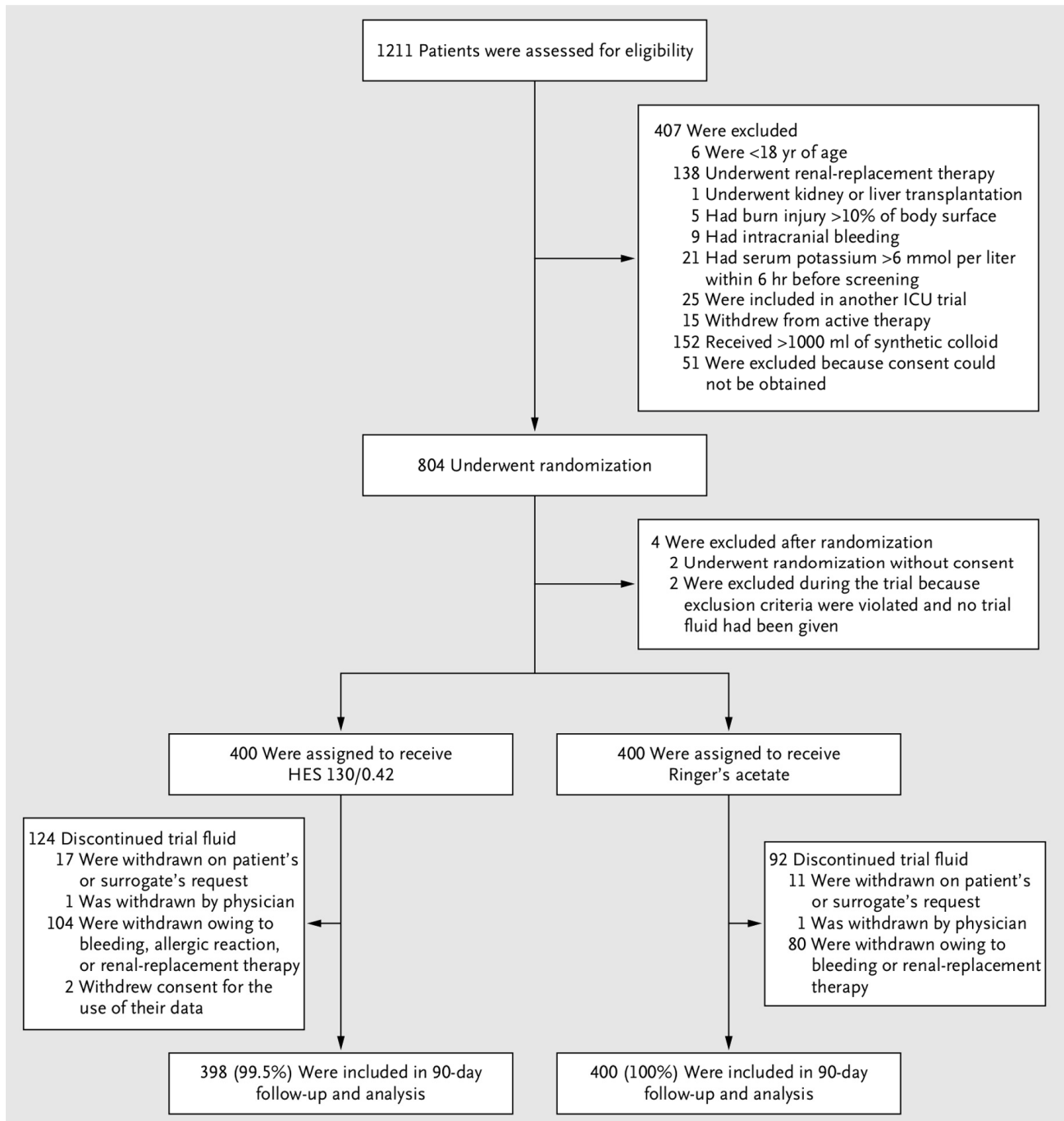

Figure 3
CONSORT diagram. From [27]. Copyright © (2012) Massachusetts Medical Society. Reprinted with permission.

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.

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 Sequential Organ Failure Assess-

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

Table 1
Primary and secondary outcomes. From [27]. Copyright © (2012) Massachusetts Medical Society. Reprinted with permission.

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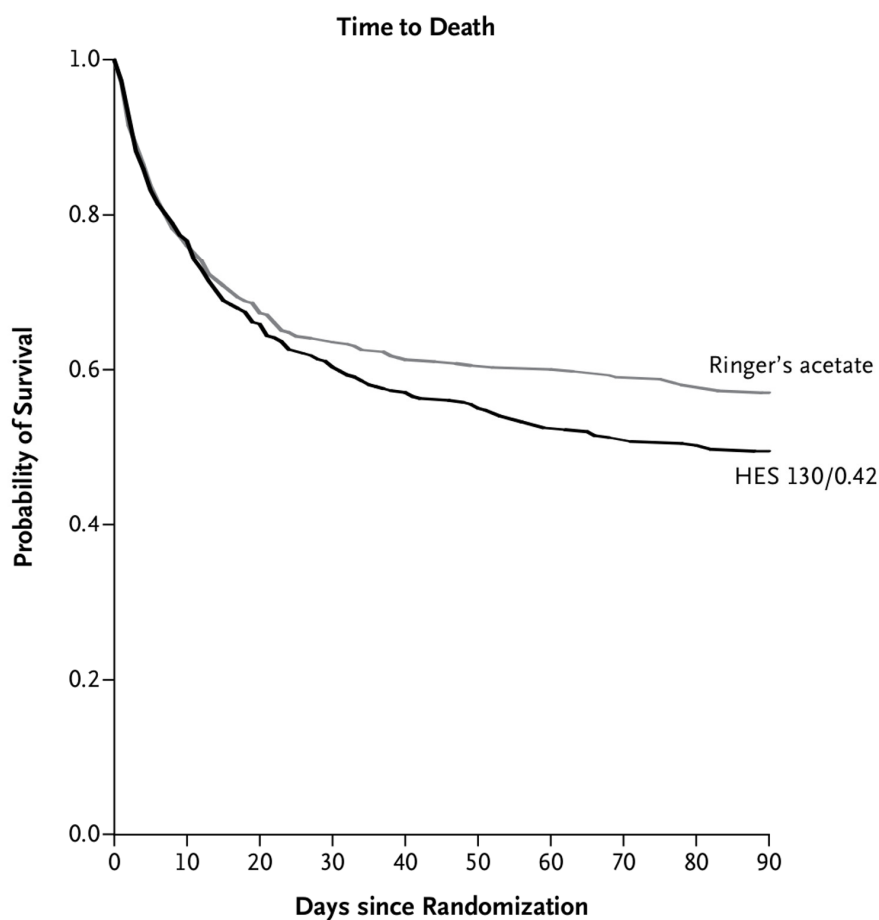
No. at Risk				
HES 130/0.42	398	240	209	197
Ringer's acetate	400	254	240	228

Figure 4
Survival curves censored at day 90 for the 798 patients in the modified population. Kaplan-Meier analysis showed that the survival time did between the two intervention groups (P=0.07). From [27]. Copyright © (2012) Massachusetts Medical Society. Reprinted with permission.

ment (SOFA) score [32]) and doubling of creatinine did not differ significantly between the groups, but the estimates favoured Ringer's acetate.

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 sequential organ failure assessment (SOFA) score 5 days after randomisation (table 1).

Post-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.

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 1.55, 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 the duration (median 1 day) and corresponding estimated blood loss were 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).
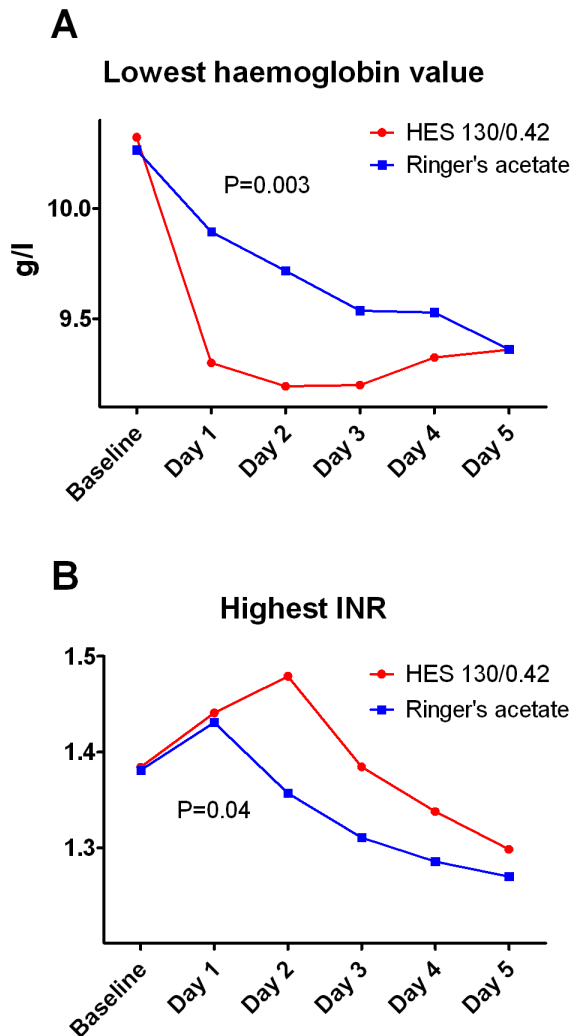
A

Lowest haemoglobin value

B

Highest INR

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. From [28].

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.

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.

6. 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 [33] and a pre-published protocol [34].

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.
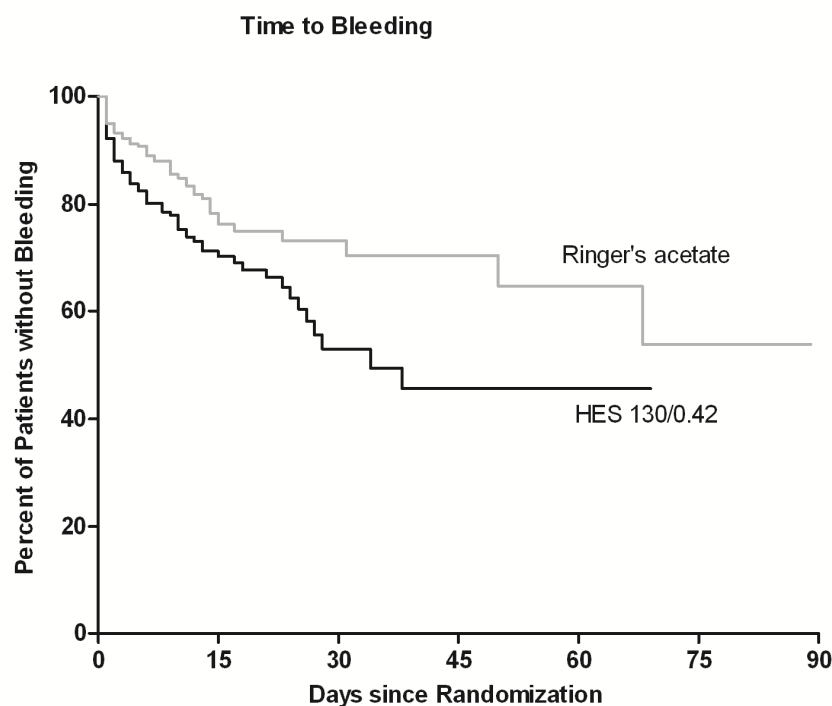
Time to Bleeding

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). From [28].

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 from one trial [27, 35–42]. 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 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.

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.
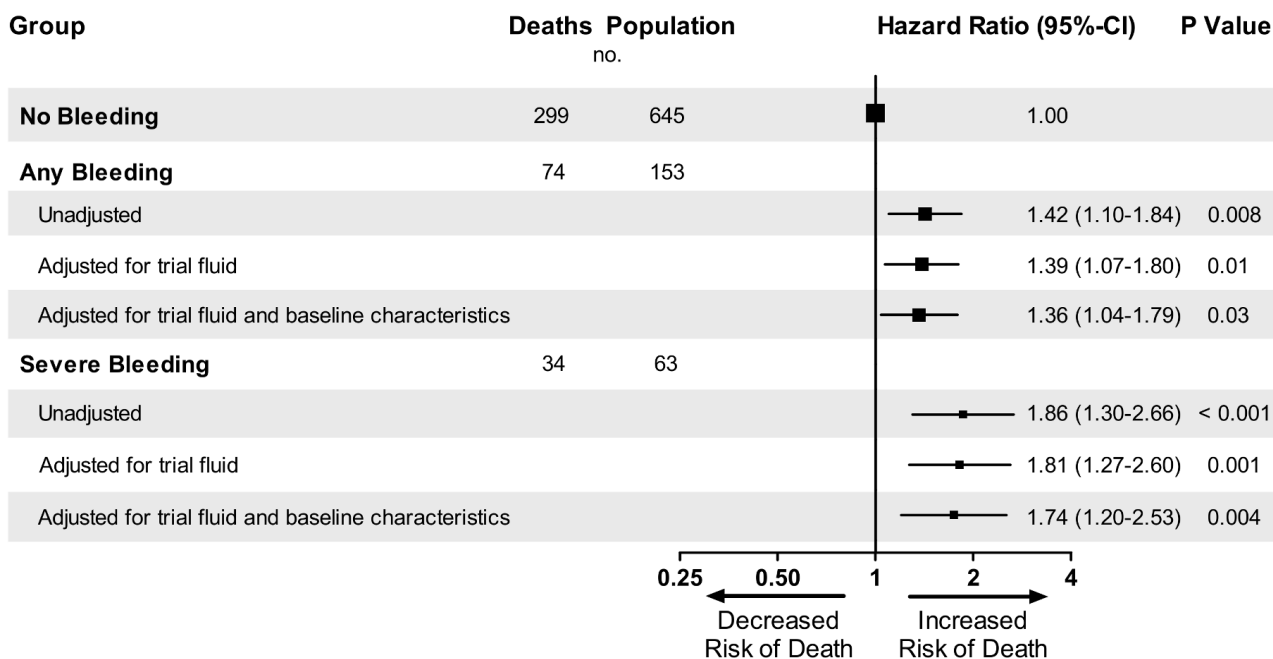
Group	Deaths	Population no.	Hazard Ratio (95%-CI)		P Value
No Bleeding	299	645	■	1.00	
Any Bleeding	74	153			
Unadjusted			■	1.42 (1.10-1.84)	0.008
Adjusted for trial fluid			■	1.39 (1.07-1.80)	0.01
Adjusted for trial fluid and baseline characteristics			■	1.36 (1.04-1.79)	0.03
Severe Bleeding	34	63			
Unadjusted			■	1.86 (1.30-2.66)	< 0.001
Adjusted for trial fluid			■	1.81 (1.27-2.60)	0.001
Adjusted for trial fluid and baseline characteristics			■	1.74 (1.20-2.53)	0.004

0.25   0.50   1   2   4

← Decreased Risk of Death   Increased Risk of Death →

Figure 7
Hazard ratio for death according to occurence of any bleeding or severe bleeding. From [28].

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.

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.

7. 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 [33, 43].

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 [44]. 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.

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

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).

Table 2
Results from the conventional meta-analysis and trial sequential analysis (TSA). Blood loss and transfused volume of blood did not differ between the groups (data not shown).

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 [45]. 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 explains 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, 46], 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 [47, unpublished manuscript seen for peer-review]. However, patients present 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 failure was a contraindication for tetrastarch in few ICUs only, while other ICUs considered acute kidney failure a specific indication for tetrastarch [26].

Secondly, the included patients might already be 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 [48–51]. In post-hoc analyses stratifying patients according to circulatory parameters and fluid given prior 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.
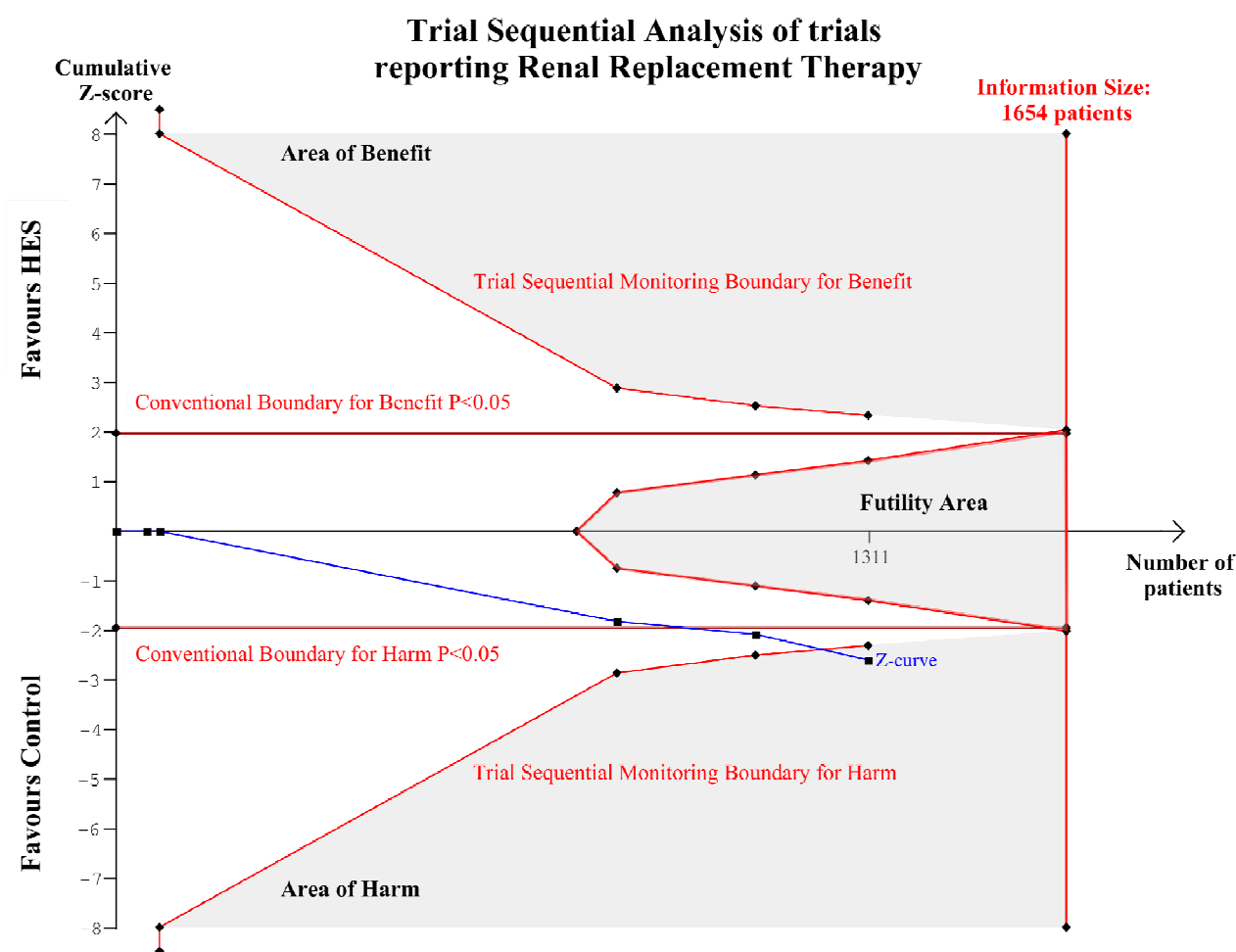
Trial Sequential Analysis of trials reporting Renal Replacement Therapy

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.

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 [48–52].

Overdosing of HES hampered the interpretation of the previous VISEP trial [53]. In our trial, some patients received more trial fluid than protocolized, but because the protocolized 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 [54]. 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 thresholds for initiation of renal replacement therapy and in use of fluids and blood products. The secondary outcomes may be considered as surrogates, which should be interpreted with caution, as they 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 [55]. 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 [56, 57].

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 [31], 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 correlate patients in the same stratum, and ignoring this correlation may lead to too wide 95% confidence intervals and a reduction in power [58, 59]. 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 [60], and some argue that they should be omitted.

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. after conversion of an atrial flutter [61]. 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 [61], 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 patients and relatives may either wish to stop the ongoing trial or deny use of data. This may lead to loss of 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 [62]. 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 / 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 [63, 64].

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 [65]. 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 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[66]. 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 [67, 68]. 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 [33]

and reporting according to the PRISMA guidelines [69]. 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 [33]. 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 [70].

Trial Sequential Analysis
Another strength of our review was the application of trial sequential analysis, because conventional meta-analysis may produce random errors due to sparse data and repetitive testing of accumulating data [71, 72]. 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 [71, 73–75]. 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 [76, 77]. 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 [78].

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 [79–81].

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=0.054) 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 regards 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 our results, 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 [35]. In this high-quality pragmatic trial 7000 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 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) than in the saline group. 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 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 3000 critically ill ICU patients to open-labelled resuscitation with HES, albumin or any other colloid vs. any crystalloid solution, but is not yet published [82]. 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 [83]. 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 [84, 85]. 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 [84]).

Two other trials using older starches in trauma did not provide evidence for safe use of HES in these patients [86, 87].

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, 81].

The volume effect of HES in surgery is poorly investigated, because only few trials were left comparing the potency of tetras-

tarch vs. crystalloid after the retraction of the studies by Boldt. There may be early circulatory benefits with tetrastarch vs. crystalloid [88, 89], but this has not been a consistent finding [90]. In surgical patients, the volume effect of 1 litre of tetrastarch seems equalled by 1 to 1.5 litre of crystalloid, which is comparable to findings in critically ill patients [27, 29, 35].

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 [91]. 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 [92].

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) [93], prolonged prothrombin time [94] and impaired thrombelastometric parameters [93–97], but only one trial reported significantly increased blood loss during surgery [98]. 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 [99, 100]. 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 polymerization [95–97, 101–103]. 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 polymerization 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 (paper II).

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 [101, 104].

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 seems 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 [105], but other concomitant interventions have not yet been identified.

8. CONCLUSION AND FUTURE PERSPECTIVES

The 6S trial is one of several high-quality clinical trials in septic and other critically ill patients 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 attempts to find a certain subgroup of patients with an overall benefit of HES beyond surrogate parameters have failed.

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 [106]. As marketing authorisations of medical products are harmonized across countries in the European Union, all member states will have to review this recommendation before it can be implemented. 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 causes more harm than good, and who can live with that?

9. 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.

10. REFERENCES

1. Fresenius Annual Report 2011. http://www.fresenius.com/documents/F_GB11_2011_e _Internet.pdf.
2. Levy MM, Fink MP, Marshall JC et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Crit Care Med 2003; 31:1250–6.
3. Brunkhorst FM, Engel C, Bloos F et al. Intensive insulin therapy and pentastarch resuscitation in severe sepsis. N Engl J Med 2008; 358:125–39.
4. Martin GS, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. N Engl J Med 2003; 348:1546–54.
5. Angus DC, Linde-Zwirble WT, Lidicker J et al. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit Care Med 2001; 29:1303–10.
6. Abraham E, Singer M. Mechanisms of sepsis-induced organ dysfunction. Crit Care Med 2007; 35:2408–16.
7. Banks W, Greenwood CT, Muir DD. The structure of hydroxyethyl starch. Br J Pharmacol 1973; 47:172–8.
8. Westphal M, James MFM, Kozek-Langenecker S et al. Hydroxyethyl starches: different products--different effects. Anesthesiology 2009; 111:187–202.
9. Jungheinrich C, Neff TA. Pharmacokinetics of hydroxyethyl starch. Clin Pharmacokinet 2005; 44:681–99.
10. Bellmann R, Feistritzer C, Wiedermann CJ. Effect of molecular weight and substitution on tissue uptake of hydroxyethyl starch: a meta-analysis of clinical studies. Clin Pharmacokinet 2012; 51:225–36.
11. Fink M, Abraham E, Vincent JL, Kochanek P. Textbook of Critical Care, 5th edition, Elsevier Saunders, 2005.
12. Boyd JH, Forbes J, Nakada T et al. Fluid resuscitation in septic shock: a positive fluid balance and elevated central venous pressure are associated with increased mortality. Crit Care Med 2011; 39:259–65.
13. Bellomo R, Cass A, Cole L et al. An observational study fluid balance and patient outcomes in the Randomized Evaluation of Normal vs. Augmented Level of Replacement Therapy trial. Crit Care Med 2012; 40:1753–60.
14. Schortgen F, Lacherade JC, Bruneel F et al. Effects of hydroxyethylstarch and gelatin on renal function in severe sepsis: a multicentre randomised study. Lancet 2001; 357:911–6.
15. Wilkes MM, Navickis RJ, Sibbald WJ. Albumin versus hydroxyethyl starch in cardiopulmonary bypass surgery: a meta-analysis of postoperative bleeding. Ann Thorac Surg 2001; 72:527–33; discussion 534.
16. Kimme P, Jannsen B, Ledin T et al. High incidence of pruritus after large doses of hydroxyethyl starch (HES) infusions. Acta Anaesthesiol Scand 2001; 45:686–9.
17. Schmidt-Hieber M, Loddenkemper C, Schwartz S et al. Hydrops lysosomalis generalisatus--an underestimated side effect of hydroxyethyl starch therapy? Eur J Haematol 2006; 77:83–5.
18. Christidis C, Mal F, Ramos J et al. Worsening of hepatic dysfunction as a consequence of repeated hydroxyethylstarch infusions. J Hepatol 2001; 35:726–32.
19. Pillebout E, Nochy D, Hill G et al. Renal histopathological lesions after orthotopic liver transplantation (OLT). Am J Transplant 2005; 5:1120–9.
20. Heilmann L, Lorch E, Hojnacki B et al. Accumulation of two different hydroxyethyl starch preparations in the placenta after hemodilution in patients with fetal intrauterine growth retardation or pregnancy hypertension. Infusionstherapie 1991; 18:236–43.
21. Sirtl C, Laubenthal H, Zumtobel V et al. Tissue deposits of hydroxyethyl starch (HES): dose-dependent and time-related. Br J Anaesth 1999; 82:510–5.
22. Wiedermann CJ. Hydroxyethyl starch--can the safety problems be ignored? Wien Klin Wochenschr 2004; 116:583–94.
23. Reinhart K, Hartog CS, Brunkhorst FM. Hydroxyethyl starches: Plus ça change, plus c'est la même chose. Anesthesiology 2010; 112:756–7.
24. Hartog CS, Kohl M, Reinhart K. A systematic review of third-generation hydroxyethyl starch (HES 130/0.4) in resuscitation: safety not adequately addressed. Anesth Analg 2011; 112:635–45.
25. Finfer S, Liu B, Taylor C et al. Resuscitation fluid use in critically ill adults: an international cross-sectional study in 391 intensive care units. Crit Care 2010; 14:R185.
26. Preferences for colloid use in Scandinavian intensive care units. Acta Anaesthesiol Scand 2008; 52:750–8.
27. Perner A, Haase N, Guttormsen AB et al. Hydroxyethyl starch 130/0.42 versus Ringer's acetate in severe sepsis. N Engl J Med 2012; 367:124–34.
28. Haase N, Wetterslev J, Winkel P et al. Bleeding and risk of death with hydroxyethyl starch in severe sepsis – Post-hoc analyses of a randomised clinical trial. Intensive Care Med 2013.
29. Haase N, Perner A, Hennings LI et al. 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.
30. Perner A, Haase N, Wetterslev J et al. Comparing the effect of hydroxyethyl starch 130/0.4 with balanced crystalloid solution on mortality and kidney failure in patients with severe sepsis (6S--Scandinavian Starch for Severe Sepsis/Septic Shock trial): study protocol, design and rationale for a double-blinded randomised clinical trial. Trials 2011; 12:24.
31. The Scandinavian Starch for Severe Sepsis / Septic Shock (6S) Trial - Statistical Analysis Plan. http://www.ssai.info/assets/files/6S-Trial_Statistical_Analysis.pdf.
32. Vincent JL, Moreno R, Takala J et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European So-

ciety of Intensive Care Medicine. Intensive Care Med 1996; 22:707–10.

33. Higgins J, Green S. Cochrane Handbook for Systematic Reviews of Interventions, Version 5.1.0 [updated March 2011], The Cochrane Collaboration, 2011. Available from www.cochrane–handbook.org.

34. Hydroxyethyl starch 130 versus crystalloid and albumin for fluid resuscitation in septic patients: effects on mortality, kidney function and hemostasis. PROSPERO 2011:CRD42011001762. http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42011001762.

35. Myburgh JA, Finfer S, Bellomo R et al. Hydroxyethyl starch or saline for fluid resuscitation in intensive care. N Engl J Med 2012; 367:1901–11.

36. Guidet B, Martinet O, Boulain T et al. Assessment of hemodynamic efficacy and safety fluid replacement in patients with severe sepsis: The CRYSTMAS study. Crit Care 2012; 16:R94.

37. Dolecek M, Svoboda P, Kantorová I et al. Therapeutic influence of 20 % albumin versus 6% hydroxyethylstarch on extravascular lung water in septic patients: a randomized controlled trial. Hepatogastroenterology 2009; 56:1622–8.

38. Dubin A, Pozo MO, Casabella CA et al. Comparison of 6% hydroxyethyl starch 130/0.4 and saline solution for resuscitation of the microcirculation during the early goal-directed therapy of septic patients. J Crit Care 2010; 25:659.e1–8.

39. Lv J, Zhao H, Liu F, An Y. The influence of lactate Ringer solution versus hydroxyethyl starch on coagulation and fibrinolytic system in patients with septic shock. Zhongguo Wei Zhong Bing Ji Jiu Yi Xue 2012; 24:38–41.

40. Palumbo D, Servillo G, D'Amato L et al. The effects of hydroxyethyl starch solution in critically ill patients. Minerva Anestesiol ; 72:655–64.

41. Zhu G, Quan Z, Shao Y et al. The study of hypertonic saline and hydroxyethyl starch treating severe sepsis. Zhongguo Wei Zhong Bing Ji Jiu Yi Xue 2011; 23:150–3.

42. Basel Starch Evaluation in Sepsis (BaSES). http://clinicaltrials.gov/ct2/show/NCT00273728.

43. Savović J, Jones HE, Altman DG et al. Influence of reported study design characteristics on intervention effect estimates from randomized, controlled trials. Ann Intern Med 2012; 157:429–38.

44. Tunis SR, Stryer DB, Clancy CM. Practical clinical trials: increasing the value of clinical research for decision making in clinical and health policy. JAMA 2003; 290:1624–32.

45. Claessens Y-E, Aegerter P, Boubaker H et al. Are clinical trials dealing with severe infection fitting routine practices? Insights from a large registry. Crit Care 2013; 17:R89.

46. Ranieri VM, Thompson BT, Barie PS et al. Drotrecogin alfa (activated) in adults with septic shock. N Engl J Med 2012; 366:2055–64.

47. Wise R, Fourie C, Richards GA. Protocols, physiology, and trials of hydroxyethyl starch. N Engl J Med 2012; 367:1266–7; author reply 1267.

48. Ertmer C, Kampmeier T, Van Aken H. Fluid therapy in critical illness: a special focus on indication, the use of hydroxyethyl starch and its different raw materials. Curr Opin Anaesthesiol 2013.

49. Von Heymann C, Sander M, Spies CD. Protocols, physiology, and trials of hydroxyethyl starch. N Engl J Med 2012; 367:1265–6; author reply 1267.

50. Magder S. Protocols, physiology, and trials of hydroxyethyl starch. N Engl J Med 2012; 367:1265; author reply 1267.

51. Chappell D, Jacob M. Protocols, physiology, and trials of hydroxyethyl starch. N Engl J Med 2012; 367:1266; author reply 1267.

52. McCluskey SA, Karkouti K. Starches for fluid therapy: Is it time for a re-appraisal, or has the horse left the barn? Can J Anaesth 2013.

53. Bracco D, Schricker T, Carvalho G. Insulin and pentastarch for severe sepsis. N Engl J Med 2008; 358:2072; author reply 2074–5.

54. Ferreira-González I, Busse JW, Heels-Ansdell D et al. Problems with use of composite end points in cardiovascular trials: systematic review of randomised controlled trials. BMJ 2007; 334:786.

55. Ciani O, Buyse M, Garside R et al. Comparison of treatment effect sizes associated with surrogate and final patient relevant outcomes in randomised controlled trials: meta-epidemiological study. BMJ 2013; 346:f457.

56. Bagshaw SM, Uchino S, Bellomo R et al. Septic acute kidney injury in critically ill patients: clinical characteristics and outcomes. Clin J Am Soc Nephrol 2007; 2:431–9.

57. Clermont G, Acker CG, Angus DC et al. Renal failure in the ICU: comparison of the impact of acute renal failure and end-stage renal disease on ICU outcomes. Kidney Int 2002; 62:986–96.

58. Kahan BC, Morris TP. Improper analysis of trials randomised using stratified blocks or minimisation. Stat Med 2012; 31:328–340.

59. Kahan BC, Morris TP. Reporting and analysis of trials using stratified randomisation in leading medical journals: review and reanalysis. BMJ 2012; 345:e5840.

60. Deeks JJ, Dinnes J, D'Amico R et al. Evaluating non-randomised intervention studies. Health Technol Assess 2003; 7:iii–x, 1–173.

61. Fergusson D, Aaron SD, Guyatt G, Hébert P. Post-randomisation exclusions: the intention to treat principle and excluding patients from analysis. BMJ 2002; 325:652–4.

62. Jansen TC, Bakker J, Kompanje EJO. Inability to obtain deferred consent due to early death in emergency research: effect on validity of clinical trial results. Intensive Care Med 2010; 36:1962–5.

63. Pocock SJ, Ariti CA, Collier TJ, Wang D. The win ratio: a new approach to the analysis of composite endpoints in clinical trials based on clinical priorities. Eur Heart J 2012; 33:176–82.

64. Finkelstein DM, Schoenfeld DA. Combining mortality and longitudinal measures in clinical trials. Stat Med 1999; 18:1341–54.

65. Bland JM, Altman DG. Multiple significance tests: the Bonferroni method. BMJ 1995; 310:170.

66. Le Gall JR, Lemeshow S, Saulnier F. A new Simplified Acute Physiology Score (SAPS II) based on a European/North American multicenter study. JAMA ; 270:2957–63.

67. Gabriel SE, Normand S-LT. Getting the methods right--the foundation of patient-centered outcomes research. N Engl J Med 2012; 367:787–90.

68. Schafer JL. Multiple imputation: a primer. Stat Methods Med Res 1999; 8:3–15.

69. Liberati A, Altman DG, Tetzlaff J et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. BMJ 2009; 339:b2700.

70. Van Der Linden P, James M, Mythen M, Weiskopf RB. Safety of modern starches used during surgery. Anesth Analg 2013; 116:35–48.

71. Wetterslev J, Thorlund K, Brok J, Gluud C. Trial sequential analysis may establish when firm evidence is reached in cumulative meta-analysis. J Clin Epidemiol 2008; 61:64–75.

72. Higgins JPT, Whitehead A, Simmonds M. Sequential methods for random-effects meta-analysis. Stat Med 2011; 30:903–21.

73. Thorlund K, Imberger G, Walsh M et al. The number of patients and events required to limit the risk of overestimation of intervention effects in meta-analysis--a simulation study. PLoS One 2011; 6:e25491.

74. Brok J, Thorlund K, Gluud C, Wetterslev J. Trial sequential analysis reveals insufficient information size and potentially false positive results in many meta-analyses. J Clin Epidemiol 2008; 61:763–9.

75. Wetterslev J, Thorlund K, Brok J, Gluud C. Estimating required information size by quantifying diversity in random-effects model meta-analyses. BMC Med Res Methodol 2009; 9:86.

76. Hospital Presents Results of Final Report: Committee Completes Investigation in the Case of Dr Boldt. http://www.klilu.de/content/veranstaltungen___presse/pressearchiv/2012/hospital_presents_results_of_final_report_committee_completes_investigation_in_the_case_of_dr_boldt/index_ger.html.

77. Editors-in-Chief Statement Regarding irb approval of clinical trials by Joachim Boldt. http://www.aaeditor.org/EICJointStatement.pdf.

78. Reinhart K, Perner A, Sprung CL et al. Consensus statement of the ESICM task force on colloid volume therapy in critically ill patients. Intensive Care Med 2012; 38:368–83.

79. Perel P, Roberts I, Ker K. Colloids versus crystalloids for fluid resuscitation in critically ill patients. Cochrane Database Syst Rev 2013; 2:CD000567.

80. Zarychanski R, Abou-Setta AM, Turgeon AF et al. Association of hydroxyethyl starch administration with mortality and acute kidney injury in critically ill patients requiring volume resuscitation: a systematic review and meta-analysis. JAMA 2013; 309:678–88.

81. Gattas DJ, Dan A, Myburgh J et al. Fluid resuscitation with 6 % hydroxyethyl starch (130/0.4 and 130/0.42) in acutely ill patients: systematic review of effects on mortality and treatment with renal replacement therapy. Intensive Care Med 2013; 39:558–68.

82. Colloids Compared to Crystalloids in Fluid Resuscitation of Critically Ill Patients (CRISTAL) trial (NCT00318942). http://clinicaltrials.gov.

83. James MFM, Michell WL, Joubert IA et al. Resuscitation with hydroxyethyl starch improves renal function and lactate clearance in penetrating trauma in a randomized controlled study: the FIRST trial (Fluids in Resuscitation of Severe Trauma). Br J Anaesth 2011; 107:693–702.

84. Finfer S. Hydroxyethyl starch in patients with trauma. Br J Anaesth 2012; 108:159–60; author reply 160–1.

85. Reinhart K, Hartog CS. Hydroxyethyl starch in patients with trauma. Br J Anaesth 2012; 108:321–2; author reply 322–4.

86. Nagy KK, Davis J, Duda J et al. A comparison of pentastarch and lactated Ringer's solution in the resuscitation of patients with hemorrhagic shock. Circ Shock 1993; 40:289–94.

87. Carli P, Goldstein P, Lejay M et al. Remplissage vasculaire prehospitalier en traumatologie: hesteril 6% versus plasmion. Jeur. 2000; 13:101–105.

88. Feldheiser A, Pavlova V, Bonomo T et al. Balanced crystalloid compared with balanced colloid solution using a goal-directed haemodynamic algorithm. Br J Anaesth 2013; 110:231–40.

89. Lindroos A-CB, Niiya T, Silvasti-Lundell M et al. Stroke volume-directed administration of hydroxyethyl starch or Ringer's acetate in sitting position during craniotomy. Acta Anaesthesiol Scand 2013.

90. Lee JS, Ahn SW, Song JW et al. Effect of hydroxyethyl starch 130/0.4 on blood loss and coagulation in patients with recent exposure to dual antiplatelet therapy undergoing off-pump coronary artery bypass graft surgery. Circ J 2011; 75:2397–402.

91. Navickis RJ, Haynes GR, Wilkes MM. Effect of hydroxyethyl starch on bleeding after cardiopulmonary bypass: a meta-analysis of randomized trials. J Thorac Cardiovasc Surg 2012; 144:223–30.

92. Kozek-Langenecker SA, Jungheinrich C, Sauermann W, Van der Linden P. The effects of hydroxyethyl starch 130/0.4 (6%) on blood loss and use of blood products in major surgery: a pooled analysis of randomized clinical trials. Anesth Analg 2008; 107:382–90.

93. Haas T, Preinreich A, Oswald E, Pajk W. Effects of albumin 5% and artificial colloids on clot formation in small infants. Anaesthesia 2007; 62:1000–1007.

94. Jin S-L, Yu B-W. Effects of acute hypervolemic fluid infusion of hydroxyethyl starch and gelatin on hemostasis and possible mechanisms. Clin Appl Thromb Hemost 2010; 16:91–8.

95. Schramko AA, Suojaranta-Ylinen RT, Kuitunen AH et al. Rapidly degradable hydroxyethyl starch solutions impair blood coagulation after cardiac surgery: a prospective randomized trial. Anesth Analg 2009; 108:30–36.

96. Schramko A, Suojaranta-Ylinen R, Kuitunen A et al. Hydroxyethylstarch and gelatin solutions impair blood coagulation after cardiac surgery: a prospective randomized trial. Br J Anaesth 2010; 104:691–7.

97. Mittermayr M, Streif W, Haas T et al. Hemostatic changes after crystalloid or colloid fluid administration during major orthopedic surgery: the role of fibrinogen administration. Anesth Analg 2007; 105:905–17.

98. Hamaji A, Hajjar L, Caiero M et al. Volume Replacement Therapy during Hip Arthroplasty using Hydroxyethyl Starch (130/0.4) Compared to Lactated Ringer Decreases Allogeneic Blood Transfusion and Postoperative Infection. Rev Bras Anestesiol 2013; 63:27–44.

99. Lv R, Zhou Z-Q, Wu H-W et al. Hydroxyethyl starch exhibits antiinflammatory effects in the intestines of endotoxemic rats. Anesth Analg 2006; 103:149–55, table of contents.

100. Schick MA, Isbary JT, Stueber T et al. Effects of crystalloids and colloids on liver and intestine microcirculation and function in cecal ligation and puncture induced septic rodents. BMC Gastroenterol 2012; 12:179.

101. Godier A, Durand M, Smadja D et al. Maize- or potato-derived hydroxyethyl starches: is there any thromboelastometric difference? Acta Anaesthesiol Scand 2010; 54:1241–7.

102. Fenger-Eriksen C, Anker-Møller E, Heslop J et al. Thrombelastographic whole blood clot formation after ex vivo addition of plasma substitutes: improvements of the induced coagulopathy with fibrinogen concentrate. Br J Anaesth 2005; 94:324–9.

103. Fenger-Eriksen C, Tønnesen E, Ingerslev J, Sørensen B. Mechanisms of hydroxyethyl starch-induced dilutional coagulopathy. J Thromb Haemost 2009; 7:1099–105.

104. Matsota P, Politou M, Kalimeris K et al. Do different substitution patterns or plant origin in hydroxyethyl starches affect blood coagulation in vitro? Blood Coagul Fibrinolysis 2010; 21:448–51.

105. Hébert PC, Wells G, Blajchman MA et al. A multicenter, randomized, controlled clinical trial of transfusion requirements in critical care. Transfusion Requirements in Critical Care Investigators, Canadian Critical Care Trials Group. N Engl J Med 1999; 340:409–17.

106. PRAC recommends suspending marketing authorisations for infusion solutions containing hydroxyethyl-starch. http://www.ema.europa.eu/docs/en_GB/document_library/Referrals_document/Solutions_for_infusion_containing_hydroxyethyl_starch/Recommendation_provided_by_Pharmacovigilance_Risk_Assessment_Committee/WC500144448.pdf.