Protocol

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

Protocol for: 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. DOI: 10.1056/NEJMoa1204242.

This supplement contains the following items:

1. Original protocol (no changes made during trial)

2. Original statistical analysis plan, final statistical analysis plan, summary of changes

Effects of hydroxyethyl starch 130/0.4 compared with balanced crystalloid solution on mortality and kidney failure in patients with severe sepsis

The 6S Trial - Scandinavian Starch for Severe Sepsis/Septic Shock Trial

Principal investigator & Sponsor Anders Perner

EudraCT no. 2009-010104-28

Clinicaltrials.gov. NCT00962156

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Summary points

- By tradition colloids are used to obtain fast circulatory stabilisation in critically ill in general, and the use of hydroxyethyl starch (HES) may be supported by meta-analyses
- High molecular weight HES may, however, cause acute kidney failure in patients with severe sepsis
- Now the low molecular weight HES 130/0.4 is the preferred colloid in Scandinavian intensive care units (ICU) and 1st choice fluid for patients with severe sepsis
- HES 130/0.4 is largely unstudied in patients with severe sepsis
- The proposed Scandinavian multicentre trial will be conducted to assess the effects of HES 130/0.4 on mortality and end-stage kidney failure in patients with severe sepsis
- The trial will provide important data to all clinicians who resuscitate septic patients

Background

Fluid is the mainstay treatment for resuscitation of patients with severe sepsis. By tradition, colloids have been used to obtain fast circulatory stabilisation, but there are only few trials with patient-centred outcome measures on fluid resuscitation of septic patient. The Surviving Sepsis Campaign recommends either colloids or crystalloids (1), but high molecular weight hydroxyethyl starch (HES) may cause acute kidney failure (AKF) in patients with severe sepsis as observed in a recent meta-analysis (2). The three largest trials in this analysis studied HES 200/0.6 (MW in kDa/substitution ratio - hydroxyethyl groups per glucose), but found divergent results with respect to kidney failure with this starch (3, 4, 5). All these trials had methodological weaknesses (6, 7), and two large cohort studies in ICU patients also showed divergent results with respect to the risk of adverse renal effects of starch treatment (8, 9).

If HES contributes to AKF in severe sepsis, this is of importance as AKF is an independent risk factor for death in these patients (10, 11, 12, 13). And if AKF progress to end-stage kidney disease, prolonged renal replacement therapy will inflict burden to patients and society.

High molecular weight HES also causes coagulopathy and bleeding and increase the rate of transfusion during major surgery (14), but effects in ICU patients are largely unstudied.

Two Cochrane meta-analyses have been published on colloid use in critically ill in general. One compared colloids with crystalloids (15), but there were few trials on HES, so reliable conclusions cannot be drawn. The other analysis included a comparison between albumin and high molecular weight HES. In this, a relative risk reduction (RRR) greater than 20% could be rejected, but the observed 14% RRR with the use of HES could not (16). As the effects of albumin and crystalloids are likely to be equal (15), an alternative hypothesis may be that high molecular weight HES reduces the risk of death by 10 – 20% compared to crystalloids.

However the high molecular weight HES is hardly ever used in Scandinavian ICUs, where HES 130/0.4 is the preferred colloid (17) and 1st choice fluid for patients with severe sepsis (preliminary data from the SAFE TRIP study, S Finfer, personal communication, and the East Danish Septic Shock Cohort, A Perner, personal communication).

Presently there are very limited data on the effects of HES 130/0.4 in ICU patients. To our knowledge only a single study has been published, in which 20 septic patients were randomised to fluid resuscitation with HES 130/0.4 or albumin (18). As for the effects on coagulation and bleeding, these may be less pronounced for HES 130/0.4 compared to HES 200/0.6, but this has only been shown perioperatively (14).

Taken together, two hypotheses can be put forward. Resuscitation with high molecular weight HES may cause AKF in patients with severe sepsis, or may improve survival by up to 20% when compared to crystalloids. In any case, the low molecular weight HES 130/0.4 widely used is largely unstudied in septic patients. Therefore, there is an urgent need for trials on HES 130/0.4 in sepsis.

Aims
To assess the effects of HES 130/0.4 compared with a balanced crystalloid solution on mortality and end-stage kidney failure in patients with severe sepsis.

Design
Multicentre, randomised, double-blinded trial with concealed allocation of septic patients 1:1 to fluid resuscitation using 6% HES 130/0.4 in Ringers acetate (Tetraspan, B Braun Medical) or Ringers acetate (Ringerfundin, B Braun Medical) stratified by the presence of shock or not (8), haematologic malignancy or not (8) and inclusion in a university hospital or not.

Tetraspan
Tetraspan contains hydroxyethylated starch 60 mg 130/0.42 per ml and the isotonic electrolyte solution of sodium chloride, potassium chloride, calcium chloride, magnesium chloride, sodium acetate and malic acid. It is marketed for the indication 'treatment of imminent or manifest hypovolaemia and shock' in all the Nordic countries (Summary of Product Characteristics for Tetraspan 6%, B Braun Medical).

Ringerfundin
Ringerfundin contains the isotonic electrolyte solution of sodium chloride, potassium chloride, calcium chloride, magnesium chloride, sodium acetate and malic acid. It is marketed for the indication 'treatment of extracellular fluid loss by isotonic dehydration associated with manifest or imminent acidosis' in all the Nordic countries (Summary of Product Characteristics for Ringerfundin, B Braun Medical).

Inclusion
All adult patients who
- Undergo **resuscitation in the ICU**

- AND fulfilment within the previous 24 hours of the criteria for **severe sepsis** according to the Society of Critical Care Medicine/American College of Chest Physicians (SCCM/ACCP), see Appendix 1 (19)

- AND **consent is obtainable** either from the patient or by proxy (physician and/or next of kin)

The following patients will not be evaluated for inclusion:
- Age < 18 years
- Previously randomised in the 6S trial
- Allergy towards hydroxyethyl starch or malic acid
- Treatment with > 1000 ml's of any synthetic colloid within the last 24 hours prior to randomisation
- Any form of renal replacement therapy
- Acute burn injury > 10% body surface area
- Severe hyperkalaemia, p-K > 6 mM
- Liver or kidney transplantation during current hospital admission
- Intracranial bleeding within current hospitalisation
- Enrolment into another ICU trial of drugs with potential action on circulation, renal function or coagulation

- Withdrawal of active therapy

Randomisation
Staff at trial sites will have access to phone-based randomisation around the clock (CTU) to allow immediate and concealed allocation and treatment with trial fluid. Each patient will be given a unique patient-number and a randomisation number.

Primary outcome measure
The composite outcome measure of 90-day mortality or end-stage kidney disease defined as dialysis-dependency 90 days after randomisation (20) will be the primary outcome measure, and these two outcome measures will also be analysed separately.

Secondary outcome measures
- Twenty-eight-day, 6-month and 1-year mortality
- Sepsis-related organ failure assessment (SOFA, modified from (21), see Appendix 5) score (excluding Glasgow Coma Score) at day 5 after randomisation
- Kidney failure (SOFA score > 2 in the kidney component) at any time in the ICU after randomisation
- Development of kidney failure (doubling of p-creatinine values) in the ICU after randomisation
- Development of acidosis as the lowest recorded pHa in the ICU after randomisation
- Need of dialysis/haemofiltration
- Need of ventilation
- Days alive without dialysis/haemofiltration in 90 days after randomisation
- Days alive without ventilation in 90 days after randomisation
- Hospital length of stay for survivors sanctioned at 90 days after randomisation

Interventions
Trial fluid is to be used for volume expansion during the entire ICU-stay for a maximum of 90 days. The treatment will follow the recommendations for fluid therapy given by the Surviving Sepsis Campaign, see Appendix 2 (1). The maximum dose of HES 130/0.4 recommended by the manufacturers is 50 ml/kg ideal BW/24 hours. The maximum dose of trial fluid will be 33 ml/kg ideal BW/24 hours as a recent retrospective study indicated that a dose of HES 130/0.4 above this might increase the risk of kidney failure in septic patients (22). After that unmasked treatment with Ringers acetate will be given to all patients. Maintenance fluids and nutrition should be given as clinically indicated. Blood products should be given for specific indications only as recommended by the Surviving Sepsis Campaign, see Appendix 2 (1). The treating clinicians will decide all other interventions.

The following will be done to reduce the risk of giving too high doses of trial fluid:
- The maximum daily dose of trial fluid will be 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 will be reduced to the nearest 500 ml.
- On the 1st day of the trial, any synthetic colloids given 24 hours prior to randomisation will be subtracted the calculated dose of trial fluid.

If the patient is discharged and then readmitted to the ICU within 90 days of randomisation the allocated trial fluid must be used if volume expansion is indicated.

Blinding
The trial fluid is visually identical and will be delivered in identical 500 ml ‘flexibag’ plastic bottles, which will be put in black plastic bags and sealed by trial personnel not involved in randomisation or treatment of patients. A computer program (CTU) will generate the coding list with the numbers

for the bottle. At randomisation, the computer program (CTU) will allocate numbered bottles from the specific trial site to the patient. Each trial site will have sufficient number of bottles of trial fluid to be allocated to patients included. This will ensure that the patient is resuscitated only with the trial fluid that he/she was randomised to receive.

Safety
Patients will be withdrawn from the trial fluid-treatment protocol if
- Renal replacement therapy is commenced for acute kidney failure OR
- SARs or SUSARs occur (see below)

Patient withdrawn from the trial fluid-treatment protocol for the above reasons will receive open-label saline or Ringers lactate for volume expansion for the remaining days of the 90-day study period.

The treating clinician can withdraw a patient from the trial fluid-treatment protocol if
- The clinical status of the patient requires open-label fluid treatment.

The independent Data Monitoring and Safety Committee – DMSC – will recommend pausing or stopping the trial if
- Group-difference in the primary outcome measure is found at the interim analysis
- Group-difference in 28- or 90-day mortality is found at the interim analysis
- Group-difference in SARs or SUSARs is found at the interim analysis
- Results from other trials show clear benefit or harm with one of the trial fluids

Serious adverse reactions
The serious adverse reactions - SARs - described with the use of the trial fluids are allergic reactions (both) and bleeding (starch) (Tetraspan and Ringerfundin Summaries of Product Characteristics). The occurrence of these will be recorded daily on the eCRF during the ICU stay and compared for the two trial groups by the DMSC at the interim analysis. An independent statistician will prepare the data for this. During the trial, Sponsor will send a monthly report on the occurrence of SARs to the DMSC and a yearly report to the ethics committees.

Suspected unexpected serious adverse reactions (SUSARs) will be defined as serious adverse reactions not described in the Summaries of Product Characteristics for Tetraspan and Ringerfundin. SUSARs will be reported by trial site investigators to Sponsor through the eCRF within 24 h. Sponsor will report any SUSARs within 7 days to the drug agency via EudraVigilance, to the DMSC, which may request the randomisation status of the patient, and to B. Braun Medical. During the trial, Sponsor will send a monthly report on the occurrence of SUSARs to the DMSC and a yearly report to the ethics committees.

Serious adverse events (SAEs) will not be recorded as an entity, because the majority of septic ICU patients will experience SAEs during their critical illness. The SAEs will be captured in the secondary outcome measures.

Patient withdrawal
Patients who are withdrawn from the trial fluid-treatment protocol (see Safety) will be followed up and analysed as the remaining patients.

Patients may be withdrawn from the trial at any time if consent is withdrawn by the person(s), who has given surrogate consent or by the patient. The person making the withdrawal will be asked for permission to use data obtained prior to withdrawal and to obtain data for the primary outcome measure. If this is achieved the patient will be included in the final analyses. If this person declines,

all data from that patient will be destroyed and a new patient will be randomised to obtain the full sample size.

Patients who are transferred to another ICU will be withdrawn from the trial fluid-treatment protocol unless this new ICU is an active trial site. If so the allocated trial fluid will be used if volume expansion is indicated in the new ICU. In any case, patients that are transferred to another ICU will be followed up for the primary outcome measure.

Statistics
Analysis will be by intention-to-treat comparing the composite outcome measure of death or dialysis-dependency at 90 days in the two groups by chi-squared test and multiple logistic regression analysis adjusting for design and patient variables (stratification variables, age, diabetes, use of nephrotoxic drugs, previous renal dysfunction ('normal' p-creatinine > 100 µmol/l) (9), acute kidney failure at randomisation (kidney failure defining severe sepsis) (8) and SAPS II (9) and SOFA score (8) in the 24 h where randomisation was done (23).

2 x 400 patients will be needed to show a 20% RRR in the composite outcome measure under the assumption of a mortality of 45% (estimated from mean mortality rates in the AT III meta-analysis (24), the two groups in VISEP (4) and unpublished data from East Danish Septic Shock Cohort, A Perner, personal communication) and dialysis-dependency of 5% at 90 days (12, 13), thus 50% for the composite outcome measure, an alpha of 0.05 (two-sided) and a power of 80%. Thus a reduction in the frequency of the composite outcome measure from 50% to 40% can be shown.

An interim analysis will be performed after 400 patients. The DMSC will recommend that the trial is stopped if it finds a group-difference in primary outcome measure, mortality, SARs or SUSARs with $p \leq 0.001$ (Haybittle-Peto criteria) or otherwise find that the continued conduct of the trial clearly compromises patient safety.

Data registration
Data will be entered into the electronic web-based (ExpertMaker) case record form (eCRF) from patient notes (source) by trial or clinical personnel under the supervision of the trial site investigators. From the eCRFs the trial database (CTU) will be established. Paper CRF will be used in case of technical difficulties with the eCRF.

Technical specifications:
The sponsor supplies a standard description of all laboratory units of measurement, which have influence on the data. To the extent that a centre uses different unit of measurement, it must submit a correction list to the data centre and the sponsor and, if necessary, have its data capture module modified accordingly**.**

Pre-randomisation characteristics (all obtained from hospital notes):
National identification number, sex, age at randomisation, estimated height in cm, co-morbidities (previously admitted for heart failure or myocardial infarction Y/N, previous admitted for stroke Y/N, chronic treatment for arterial hypertension Y/N, chronic treatment for diabetes Y/N, previously admitted for asthma or chronic obstructive pulmonary disease Y/N), haematological malignancy Y/N, from where was the patient admitted to the ICU? (Emergency ward / general ward / operation theatre or recovery / via paramedic or ambulance services / other ICU this hospital / other hospital), elective or emergency surgery within current hospital admission Y/N, site of infection (pulmonary/abdominal/urinary tract/soft tissue/other), and 'normal' p-creatinine. Use of potential nephrotoxic drugs (Y/N) during current hospital admission: Gentamycin, vancomycin, Amphotericin B, polymyxins, ciclosporin A, IV contrast dye, NSAIDs and Cox-2 inhibitors.

24-hours prior to randomisation:

- Values for simplified acute physiology score (SAPS) II (25)
- Parameters for SOFA scoring not covered above: Lowest mean arterial blood pressure value and highest infusion rate of vasoactive drugs
- Volume of resuscitation fluids (crystalloids, colloids and blood products specified in ml)
- Results of blood samples (standard lab. values) for haemoglobin (lowest value), bilirubin (highest value), INR (highest value), d-dimer (highest value) and platelets (lowest value).

At randomisation (+/- 2 hours):
- Lowest values of mean arterial blood pressure, central venous pressure, central venous oxygen saturation and highest values of arterial or venous lactate concentration obtained from the ICU charges

12-hourly in the first 24 after randomisation:
- Highest and lowest values of central venous pressure, central venous oxygen saturation and arterial or venous lactate concentration obtained from the ICU charges

Daily in the first 5 days after randomisation:
- Results of morning samples of bilirubin, INR and platelets (standard lab. values).
- Parameters for daily SOFA scoring not covered above: Lowest blood pressure value and highest infusion rate of vasoactive drugs and lowest ratio of arterial oxygen tension/fraction of inspired oxygen obtained from the ICU charges

During the entire ICU stay:
- Daily volumes of trial fluid and other fluids incl. blood products, nutrition, total fluid input, urinary output and calculated fluid balance as of the ICU charges
- Daily lowest values of blood haemoglobin and arterial pH and standard base excess (point of care testing) and highest value of p-creatinine (standard lab. value)
- On mechanical invasive- or non-ventilation (as marked in the SOFA scoring day 0-5 and Y/N at 08.00 from day 6)
- Any use of potential nephrotoxic drugs as mentioned above (Y/N for every day)
- Bleeding episodes noted in patient files including gastrointestinal (haematemesis, frank blood or "coffee grounds" in a nasogastric aspirate, or melaena or frank blood in stools), wounds, during surgery or frank blood in urine or tracheal aspirates.
- Serious adverse reactions (Y/N for every day) including severe bleeding (intracranial bleeding or bleeding episode (defined as above) with the need for > 3 units of blood per day (defined as the 24 h of the units fluid charge)) or serious allergic reactions defined as urticaria associated with worsened circulation (20% decrease in blood pressure or 20% increase in vasopressor dose), increased airway resistance (20% increase in the peak pressure on the ventilation or clinical stridor or bronchospasme or treatment with bronchodilators).

90 days after randomisation:
- Survival status obtained from hospital or civil registries
- If the patient is deceased, date of death
- Dialysis-dependency at day 90 defined as need of a dialysis treatment session within the time period 4 days prior to or after day 90 post-randomisation as obtained from hospital notes or registries.
- Total days of dialysis-dependency (haemo-dialysis or –filtration) summarised at day 90 from hospital notes or registries. First and last treatment session will define length of dialysis-dependency in each patient.
- Date of hospital discharge as obtained from hospital notes or registries

½- and 1 year after randomisation:

- Survival status obtained from hospital or civil registries

Data handling and retention

Data will be handled according to the data protection agencies of the different countries. All original records (incl. consent forms, CRFs, SAR reports and relevant correspondences) will be retained at trial sites or CTU for 15 years to allow inspection by the GCP Unit or local authorities. The study database will be maintained for 15 years and anonymised if requested by the authorities.

Monitoring

The trial will be externally monitored (the GCP unit at University of Copenhagen) to GCP standards according to the EU directive 2001/20. Trial site investigators will give access to source data according to the Clinical Trial Agreement.

Ethics

The trial will adhere to the Helsinki Declaration II and the national laws in the Nordic countries. Inclusion will start after approval by the ethical committees, drug agencies and data protection agencies in the country of the trial site and trial registration at www.clinicaltrials.gov.

Patients will only be enrolled after informed consent, but the treatment has to be initiated immediately and most patients will be unconscious and therefore included after proxy consent (physician and/or next of kin) according to national laws. The patient or the patient next of kin and/or general practitioner will be asked for delayed consent if required by national law.

The trial cannot be performed in conscious persons, as no clinically relevant model of severe sepsis exists and no conscious patients have the combination of severe infection and multiple organ failure as septic patients have.

No biological material will be collected for the trial, thus no bio-bank will be formed.

Enrolment

Patients are expected to be included from 25 Scandinavian ICUs (Denmark 10 units, Sweden 10, Norway 2, Finland 2 units and Iceland 1 unit) during a 2-year period starting November 2009. In a previous study (SAFE TRIPS) we have enrolled patients from 63 Scandinavian ICUs.
Each of the 25 units has to include 2 patients per month (holidays excluded) to finish inclusion in 2 years. Unpublished data from East Danish Septic Shock Cohort (A Perner, personal communication) indicate that Danish ICUs treat 5 - 15 patients with septic shock per month. The number of patients with severe sepsis or septic shock will be higher making it realistic to include an average of 2 patients per ICU per month.

Data analyses and publications

An independent statistician will perform the data analysis prior to the breaking of the randomisation code. Based on these masked analyses of the primary and secondary outcome measures two abstracts will be written by the Writing Committee, the randomisation code will then be opened and a final manuscript written containing the correct of the two pre-made abstracts. The manuscript will be submitted to one of the major clinical journals regardless of the results. The Steering committee will grant authorship depending on personal input (see Appendix 4) according to the Vancouver definitions. All trial sites and trial site investigators will be acknowledged. Funding sources will have no influence on data handling or analysis or writing of the manuscript.
Side studies will be allowed if supported by the Steering committee.

Timeline

2008 - 2009: Applications for funding, ethical committees and drug agencies, development of CRFs and data management tools and development of monitoring plan and education of monitors
2009 - 2011: Inclusion of patients
2010: Interim analysis
2011: Data analysis, writing of a manuscript and publication

Tasks and responsibilities
Principle Investigator (PI) and 'Sponsor': Anders Perner

Trial Steering and Management Committee: The national investigators, Nicolai Haase (PhD student at Rigshospitalet and CTU) and Jørn Wetterslev (CTU) will apply for funding and ethical and drug agency approvals and will recruit and manage trial sites in their countries.

Trial Site Investigators will be responsible for all trial-related procedures at their site including trial fluid, education of staff in trial-related procedures, recruitment and follow-up of patients and entry of data. Clinical staff at the trial sites will do the treatment of trial patients.

Independent Data Monitoring and Safety Committee: Daniel De Backer (chair), Peter Dalgaard and Kathy Rowan will evaluate SUSARs and the interim analyses and will provide recommendations about stopping or continuing the trial to the Steering Committee of the trial, see Charter for the DMSC, Appendix 3.

Collaborators
Copenhagen Trial Unit, Centre for Clinical Intervention Research, Rigshospitalet will develop the CRF together with the Steering Committee and systems for phone-based randomisation, allocation of trial fluid and data handling.

The GCP unit at University of Copenhagen will develop the monitoring plan, educate monitors and coordinate monitoring in cooperation with the GCP units in Aarhus, Lund and Oslo. Together these units will cover all trial sites in all countries.

The Scandinavian Society of Anaesthesia and Intensive Care will provide the platform for web-based data entry - the eCRF (Expertmaker AB).

B Braun Medical AG (Melsungen, Germany) will delivery trial fluids to all trial sites.

Finances
The Danish research council support the trial for 2009 - 2012 (1,2 mill Dkr.). Public and private funds will be applied for the remaining budget.

Insurance
The patients will be covered by the insurance of the trial sites.

Perspective
Severe sepsis affects millions of patients worldwide with high rates of complications and mortality. Outcome differences between therapies for severe sepsis will, therefore, have major impact on global health and healthcare costs.

Currently, two other RCTs are assessing the effect of HES 130/0.4 in sepsis:
BASES is a single centre study expected to include 250 patients with severe sepsis. The primary outcome measure is ICU length of stay, but biochemical markers of kidney failure and the frequency of renal replacement therapy is also recorded (clinicaltrial.gov identifier: NCT00273728). Inclusion is expected to be completed in May 2010 (M Siegemund, personal communication).

CRYSTMAS is a multicentre study expected to include 200 patients with severe sepsis. The primary outcome measure is amount of fluid required to achieve initial haemodynamic stabilisation and amount of enteral calories given in the 7 days after stabilisation. Biochemical markers of kidney failure will also be recorded. Inclusion is expected to be completed in April 2010 (clinicaltrial.gov identifier: NCT00464204).

In addition, an Australian/New Zealand multicentre RCT is planned to compare HES 130/0.4 and saline in 7,000 hypovolaemic ICU patients (NCT00935168). The primary outcome measure will be 90-day mortality and subgroup analysis of patients with severe sepsis is planned (J Myburgh, personal communication). We collaborate with the investigators and plan a common individual patient data meta-analysis combining our data with those of their patients with severe sepsis. Thus in- and exclusion criteria and outcome measures of the two trials have been aligned to prepare for the common analysis.

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Appendix 1

Trial criteria for severe sepsis

SEPSIS is defined as a (1) DEFINED FOCUS OF INFECTION AND (2) at least TWO systemic inflammatory response syndrome (SIRS) criteria.

(1) DEFINED FOCUS OF INFECTION is 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 are:

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

SEVERE SEPSIS is 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 is defined as a Sequential Organ Failure Assessment (SOFA) score $\geq$ 2 for the organ in question, see Appendix 5.

Appendix 2

The Surviving Sepsis Campaigns recommendations (1)

For fluid therapy

- Use a fluid challenge technique while associated with a haemodynamic improvement
- Give fluid challenges of 500 - 1000 ml over 30 min. More rapid and larger volumes may be required in sepsis-induced tissue hypoperfusion
- Rate of fluid administration should be reduced if cardiac filling pressures increase without concurrent haemodynamic improvement

For endpoints of resuscitation in the first 6 hours of severe sepsis or septic shock

- Central venous pressure: 8–12 mm Hg*
- Mean arterial pressure > 65 mm Hg
- Urine output > 0.5 ml/kg/h
- Central venous (superior vena cava) or mixed venous oxygen saturation $ScvO_2$ / SvO_2 > 70%

If venous O_2 saturation target is not achieved:
– consider further fluid
– transfuse packed red blood cells if required to haematocrit of ≥ 30% and/or
– dobutamine infusion max 20 µg/kg/min

*A higher target CVP of 12–15 mm Hg is recommended in the presence of mechanical ventilation or pre-existing decreased ventricular compliance.

For administration of blood products

- Give red blood cells when haemoglobin decreases to < 7.0 g/dl (< 4.5 mM) to target haemoglobin of 7.0 - 9.0 g/dl (4.5 – 5.6 mM). A higher haemoglobin level may be required in special circumstances (e.g.: myocardial ischaemia, severe hypoxaemia, acute haemorrhage, cyanotic heart disease or lactic acidosis)
- Do not use fresh frozen plasma to correct laboratory clotting abnormalities unless there is bleeding or planned invasive procedures
- Administer platelets when

– counts are < 5×10^9/l regardless of bleeding
– counts are 5 - 30×10^9/l and there is significant bleeding risk
– higher platelet counts (≥ 50×10^9/l) are required for surgery or invasive procedures

Appendix 3

Charter for the independent Data Monitoring and Safety Committee (DMSC) of the 6S trial.

EudraCT no. 2009-010104-28 Clinical Trial no. NCT00962156.

Copenhagen 2009

Introduction
The Charter will define the primary responsibilities of the DMSC, its relationship with other trial components, its membership, and the purpose and timing of its meetings. The Charter will also provide the procedures for ensuring confidentiality and proper communication, the statistical monitoring guidelines to be implemented by the DMSC, and an outline of the content of the Open and Closed Reports that will be provided to the DMSC.

Primary responsibilities of the DMSC
The DMSC will be responsible for safeguarding the interests of trial participants, assessing the safety and efficacy of the interventions during the trial, and for monitoring the overall conduct of the clinical trial. The DMSC will provide recommendations about stopping or continuing the trial to the Steering Committee (SC) of the 6S trial. To contribute to enhancing the integrity of the trial, the DMSC may also formulate recommendations relating to the selection/recruitment/retention of participants, their management, improving adherence to protocol-specified regimens and retention of participants, and the procedures for data management and quality control.

The DMSC will be advisory to the SC. The SC will be responsible for promptly reviewing the DMSC recommendations, to decide whether to continue or terminate the trial, and to determine whether amendments to the protocol or changes in trial conduct are required.

Members of the DMSC
The DMSC is an independent multidisciplinary group consisting of clinicians and a biostatistician that, collectively, has experience in the management of ICU patients and in the conduct, monitoring and analysis of randomised clinical trials.

DMSC Clinician
Dr. Daniel De Backer, Dept. of Intensive Care, Erasme University Hospital, Brussels

DMSC Clinical trialist
Prof. Kathy Rowan, Intensive Care National Audit and Research Center (ICNARC), London

DMSC Biostatistician
Dr. Peter Dalgaard, Dept. of Biostatistics, University of Copenhagen

Conflicts of interest
DMSC membership has been restricted to individuals free of conflicts of interest. The source of these conflicts may be financial, scientific, or regulatory in nature. Thus, neither trial investigators nor individuals employed by the sponsor, nor individuals who might have regulatory responsibilities for the trial products, are members of the DMSC. The DMSC members do not own stock in the companies having products being evaluated by the 6S trial.

The DMSC members will disclose to fellow members any consulting agreements or financial interests they have with the sponsor of the trial, with the contract research organisation (CRO) for the trial (if any), or with other sponsors having products that are being evaluated or having products that are competitive with those being evaluated in the trial.

The DMSC will be responsible for deciding whether these consulting agreements or financial interests materially impact their objectivity.

The DMSC members will be responsible for advising fellow members of any changes in these consulting agreements and financial interests that occur during the course of the trial. Any DMSC members who develop significant conflicts of interest during the course of the trial should resign from the DMSC.

DMSC membership is to be for the duration of the clinical trial. If any members leave the DMSC during the course of the trial, the SC will appoint the replacement(s).

Formal interim analysis meeting
One 'Formal Interim Analysis' meeting will be held to review data relating to treatment efficacy, patient safety, and quality of trial conduct. The three members of the DMSC will meet when 90-day follow-up data of 400 patients have been obtained.

Proper communication
To enhance the integrity and credibility of the trial, procedures will be implemented to ensure the DMSC has sole access to evolving information from the clinical trial regarding comparative results of efficacy and safety data, aggregated by treatment group (0,1). An exception will be made to permit access to an independent statistician who will be responsible for serving as a liaison between the database and the DMSC.

At the same time, procedures will be implemented to ensure that proper communication is achieved between the DMSC and the trial investigators. To provide a forum for exchange of information among various parties who share responsibility for the successful conduct of the trial, a format for Open Sessions and Closed Sessions will be implemented. The intent of this format is to enable the DMSC to preserve confidentiality of the comparative efficacy results while at the same time providing opportunities for interaction between the DMSC and others who have valuable insights into trial-related issues.

Closed Sessions
Sessions involving only DMSC membership who generates the Closed Reports (called Closed Sessions) will be held to allow discussion of confidential data from the clinical trial, including information about the relative efficacy and safety of interventions. In order to ensure that the DMSC will be fully informed in its primary mission of safeguarding the interest of participating patients, the DMSC will be blinded in its assessment of safety and efficacy data. However, the DMSC can request unblinding from the SC.

Open Reports
For each DMSC meeting, Open Reports will be provided available to all who attend the DMSC meeting. The Reports will include data on recruitment and baseline characteristics, and pooled data on eligibility violations, completeness of follow-up, and compliance. The primary trial statistician will prepare these Open Reports.

Closed Reports will include analysis of the primary efficacy outcome measure. In addition, analyses of the secondary outcome measures and serious adverse events will also be reported.

These Closed Reports will be prepared by an independent biostatistician, with assistance from the trial biostatisticians, in a manner that allow them to remain blinded.

The Closed Reports should provide information that is accurate, with follow-up on mortality that is complete to within two months of the date of the DMSC meeting.

The Reports should be provided to DMSC members approximately three days prior to the date of the meeting.

Minutes of the DMSC Meetings
The DMSC will prepare minutes of their meetings. The Closed Minutes will describe the proceedings from all sessions of the DMSC meeting, including the listing of recommendations by the Committee. Because it is likely that these minutes may contain unblinded information, it is important that they are not made available to anyone outside the DMSC.

Recommendations to the Steering Committee
After the interim analysis meeting, the DMSC will make a recommendation to the SC to continue, hold or terminate the trial.

This recommendation will be based primarily on safety and efficacy considerations and will be guided by statistical monitoring guidelines defined in this Charter and the trial protocol.

The SC is jointly responsible with the DMSC for safeguarding the interests of participating patients and for the conduct of the trial. Recommendations to amend the protocol or conduct of the trial made by the DMSC will be considered and accepted or rejected by the SC. The SC will be responsible for deciding whether to continue, hold or stop the trial based on the DMSC recommendations.
The DMSC will be notified of all changes to the trial protocol or conduct. The DMSC concurrence will be sought on all substantive recommendations or changes to the protocol or trial conduct prior to their implementation.

Statistical monitoring guidelines
The outcome parameters are defined in the 6S trial protocol.

For the two intervention groups, the DMSC will evaluate data on:

<u>The primary outcome measure</u>
The composite outcome measure of death or dialysis-dependency 90 days after randomisation analysed together and separately

<u>The secondary outcome measures</u>
28-day mortality
Severity organ failure assessment (SOFA) score at day 5 after randomisation
Kidney failure (SOFA score >2 in the kidney component) during the ICU stay
Need of dialysis
Days free of dialysis for survivors
Serious adverse reactions - SARs - (severe allergic reactions or severe bleeding) and suspected unexpected serious adverse reactions - SUSARs

The DMSC will be provided with these data from the Coordinating Centre as:
a. Number of patients randomised
b. Number of patients randomised per intervention group (0,1)
c. Number of patients stratified pr. stratification variable per intervention group (0,1)

d. Number of events, according to the outcomes, in the two groups

Based on evaluations of these outcomes, the DMSC will decide if they want further data from the Coordinating Centre and when next to perform analyses of the data.

For analyses, the data will be provided in one file as described below.

Based on the analyses of the primary outcome measure and SARs, the DMSC will use P<0.001 (Haybittle-Peto) as the statistical limit to guide its recommendations regarding early termination of the trial.

Based on 28- and 90-day mortality analyses, the DMSC will use P<0.001 (Haybittle-Peto) and group sequential monitoring boundaries as the statistical limit to guide its recommendations regarding early termination of the trial.

DMSC should also be informed about all SUSARs and SARs occurring in the two groups of the trial.

The DMSC may also be asked to ensure that procedures are properly implemented to adjust trial sample size or duration of follow-up to restore power, if protocol specified event rates are inaccurate. If so, the algorithm for doing this should be clearly specified.

Conditions for transfer of data from the Coordinating Centre to the DMSC
The DMSC shall be provided with the data described below in one file.

The DMSC will be provided with an Excel database containing the data defined as follows:

1. Row 1 contains the names of the variables (to be defined below).

2. Row 2 to N (where N-1 is the number of patients who have entered the trial) each contains the data of one patient.

3. Column 1 to p (where p is the number of variables to be defined below) each contains in row 1 the name of a variable and in the next N rows the values of this variable.

The values of the following variables should be included in the database:

1: PtID: a number that uniquely identifies the patient.

2: Rdcode: The randomisation code (group 0 or 1) – the DMSC is not to be informed on what intervention the groups received.

3. 1.EndInd: Primary outcome measure indicator (1 if patient fulfilled the primary outcome measure at day 90 and 0 if the patient did not).

4: 90MInd: 90 day-mortality indicator (2 if patient is censored, 1 if patient was dead, and 0 if the patient was alive at day 90).

5: 90DIAInd: Dialysis dependency at day 90 (2 if patient is censored, 1 if patient is dependent, and 0 if the patient is not).

6: 28MInd: 28 day-mortality indicator (2 if patient is censored, 1 if patient is dead, and 0 if the patient is known to be alive).

7: SOFAInd: SOFA indicator (SOFA score of the patient at day 5).

8: AKFInd: Acute kidney failure indicator (1 if patient had kidney failure during the ICU stay, 0 if the patient did not).

9: DIAInd: Dialysis indicator (1 if patient needed any dialysis from randomisation till day 90 and 0 if the patient did not).

10: DiaDInd: Days free of dialysis indicator (no. of day from randomisation till day 90 free of dialysis for each patient; 0 if the patient did not need dialysis; blank if the patient had died).

11: SARInd: Serious Adverse Reaction indicator (1 if patient has had a SAR during ICU stay and 0 if the patient did not).

12: SUSARInd: Suspected Unexpected Serious Adverse Reaction indicator (1 if patient has had a SUSAR during ICU stay and 0 if the patient did not).

Appendix 4

Acknowledgment of academic contribution and authorship

All trial sites including patients will be acknowledged, and all investigators at these sites will appear with their names under ‘the 6S trial investigators’ in an Appendix to the final manuscript.

The Steering Committee will grant authorship depending on personal involvement according to the Vancouver definitions. If a trial site investigator is to gain authorship, the site has to include 30 patients or more. If the site includes 60 patients or more, two authorships will be granted.

The listing of authors will be as follows: A Perner will be the first author, N Haase the second and the next authors will be the other members of the Steering Committee according to the number of included patients per country, then trial site investigators dependent on the number of included patients per site, J Wetterslev will appear as the last author and then ‘for the 6S trial investigators’.

Appendix 5

SOFA scoring (ex. GCS) - use the most deranged value recorded in the previous 24 h (21)

ORGAN SYSTEM	0	1	2	3	4	Organ scores
Respiration						
PaO_2 / FiO_2 (in mmHg)	>400	301 - 400	<301 (without respiratory support*)	101 - 200 (with respiratory support*)	≤ 100 (with respiratory support*)	
(in kPa)	>53	40 – 53	<40 (without respiratory support*)	13 – 27 (with respiratory support*)	≤ 13 (with respiratory support*)	
Coagulation Platelets (x 10^9 / l)	>150	101 - 150	51 – 100	21 - 50	≤ 20	
Liver						
Bilirubin (mg / dl)	< 1.2	1.2 – 1.9	2.0 – 5.9	6.0 – 11.9	> 12.0	
(µmol / l)	<20	20 - 32	33 – 101	102 - 204	>204	
Cardiovascular Hypotension	MAP > 70 mmHg	MAP < 70 mmHg	dopamine ≤ 5.0 (doses are given in µg / kg / minute)	dopamine > 5.0 (doses are given in µg / kg / minute)	dopamine > 15.0 (doses are given in µg / kg / minute)	
			or any dose dobutamine	or adrenalin ≤ 0.1	or adrenalin >0.1	
			or any dose milrinone or any dose levosimendan	or noradrenalin ≤ 0.1 or any dose vasopressin or any dose phenylephrine	or noradrenalin >0.1	
Renal Creatinine (mg / dl)	< 1.2	1.2 – 1.9	2.0 – 3.4	3.5 – 4.9	> 5.0	
(µmol/l)	< 110	110 – 170	171 – 299	300 – 440	> 440	
OR Urine output				or < 500 ml / day	or < 200 ml / day	

If a value has not been measured, the score 0 should be given

*Respiratory support is defined as any form of invasive or non-invasive ventilation including mask CPAP or CPAP delivered through a tracheotomy

6S-trial - Original Statistical Analysis Plan

Populations

Intention-to-treat: All randomised patients. This population is not analysed in the 6S-trial.

Modified intention-to-treat:

All randomised patients except patients who

- were not eligible for randomisation according to the inclusion/exclusion criteria

AND

- who never had any of the interventions (masked trial fluid).

Per-protocol #1:

All randomised patients except patients having one or more major protocol violations defined as

- Patients who were not eligible for randomisation 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 randomisation.

OR

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

Per-protocol #2:

All randomised patients except patients having one or more major protocol violations defined as

- Patients who were not eligible for randomisation 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 randomisation.

OR

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

Subgroups:

- Patients where renal failure define severe sepsis (renal component of SOFA-score = 2 OR higher)
- Patients with shock at time of randomisation (mean arterial pressure < 70 mmHg after the initial fluid resuscitation OR ongoing treatment with noradrenalin, adrenalin, dopamin, dobutamin, vasopressin, phenylephrine, milrinone or levosimendan OR arterial or venous lactate > 4.0 mmol/L within the last hour)

Analyses

Primary analysis:

Unadjusted Chi-square test for binary outcome measures. For rate data the generalized linear model (SAS proc genmod) will be used with distribution Poisson, link=log and offset.

Secondary analysis (will only be made for the modified intention-to-treat population):

Multiple (logistic) regression and analysis of rate data with the following covariates:

Binary covariates
- Center is a university hospital Y/N (stratification variable)
- Diagnose of hematological malignancy at time of randomisation Y/N (stratification variable)
- Shock at time of randomisation Y/N (as defined above) (stratification variable)
- Diabetes at time of randomisation Y/N
- Use of nephrotoxic drugs during current admission and prior to randomisation Y/N
- Previous renal dysfunction ('normal' creatinine >100 µmol/l = baseline variable #2) Y/N
- Acute Kidney Failure at randomisation (renal failure defining severe sepsis as defined above)

Continuous covariate
- Age

Ordinal covariates
- SAPS II
- SOFA-score

Outcomes

Primary outcome measure
The composite outcome measure of 90-day mortality or end-stage kidney disease defined as dialysis-dependency 90 days after randomisation (+/- 4 days) as retrieved from the National Patient Hospital Register and the National Dialysis Database.

These two outcome measures will also be analysed separately.

Secondary outcome measures

- Twenty-eight-day, 6-month and 1-year mortality
- Time to death or censoring
- SOFA-score without cerebral component (see Appendix 5) on day 5 after randomisation.
- Development of kidney failure defined as
  - Renal component of SOFA-score (renal-SOFA) = 3 or higher at any time in the ICU after randomisation, but renal-SOFA < 3 before randomisation (baseline values)

  OR
  - Patient requiring dialysis at any time after randomisation
- Development of kidney failure at any time in the ICU after randomisation defined as
  - Doubling of p-creatinine values = 2 x 'normal creatinine' (baseline variable #2)
- Development of acidosis (pHa < 7,35) in the ICU after randomisation
  - As data from day 1 may reflect baseline characteristics, the analysis will be made with and without day 1.
- Need of dialysis/haemofiltration at any time after randomisation
- Need of ventilation at any time after randomisation
- Days alive without dialysis/haemofiltration in the 90 days after randomisation

  We define the value of a "period of dialysis" which runs from the day where dialysis is initiated till the day where the last dialysis is performed as the number of dates included in the period. The outcome measure is calculated as 90 days minus "the number of days in the period of dialysis" / 90 days.
- Days alive without ventilation in 90 days after randomisation

  If the patient is on the ventilator at 8 am, the day is a ventilator-day. The outcome measure is calculated as 90 days minus the number of "ventilator days" / 90 days.
- Hospital length of stay for survivors censored at 90 days after randomisation

Level of statistical significance for all analyses: P = 0.05

Missing Data

Kidney failure at time of randomisation: If the patient doesn't have kidney failure on day 1, this observation will be carried backward.

‘Normal’ creatinine < 100 mmol/l: If the patient has any creatinine < 100 mmol/l without renal replacement therapy during or after the ICU-stay, the ‘normal’ creatinine will be considered < 100 mmol/l.

SOFA-score: There will be missing data for patients who

- die before day 5

OR

- are discharged from the ICU before day 5 and are still alive on day 5.

Initially, we will perform a complete case-analysis. Then a supplementary analysis where patients who die before day 5 get the maximum score (20 points), and where patients who are discharged from the ICU and alive on day 5 will get 0 points.

For patients, who are still in the ICU on day 5, last observation will be carried forward.

If the frequency of missing data after the above implemented “imputations” is > 5% and the complete case analysis is significant at the 10% value or less, we will perform an additional analysis using the multiple imputation method.

To put significant results into perspective the following sensitivity analysis will be conducted: We define a worst case scenario as one where patients with missing data do not react on the treatment (whatever it may be). Missing data will be imputed according to this scenario. Let P be the estimate of the parameter reflecting the effect of the intervention calculated from the complete case analysis and P-imp be the corresponding estimate calculated from the analysis of the imputed data.
[(P-imp – P)/P-imp]*100% then a ball park figure of the bias is to be expected were the worst case scenario true.
P-imp/ (standard error of P-imp) is calculated and the corresponding p value found to assess the potential impact of this bias on the significance level.

6S-trial - Final Statistical Analysis Plan

Populations

Intention-to-treat: All randomised patients. This population is not analysed in the 6S-trial.

Modified intention-to-treat:

All randomised patients except patients who

- were not eligible for randomisation according to the inclusion/exclusion criteria

AND

- who never had any of the interventions (masked trial fluid).

Per-protocol #1:

All randomised patients except patients having one or more major protocol violations defined as

- Patients who were not eligible for randomisation 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 randomisation.

OR

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

Per-protocol #2:

All randomised patients except patients having one or more major protocol violations defined as

- Patients who were not eligible for randomisation 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 randomisation.

OR

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

Subgroups:

- Patients where renal failure define severe sepsis (renal component of SOFA-score = 2 OR higher)
- Patients with shock at time of randomisation (mean arterial pressure < 70 mmHg after the initial fluid resuscitation OR ongoing treatment with noradrenalin, adrenalin, dopamin, dobutamin, vasopressin, phenylephrine, milrinone or levosimendan OR arterial or venous lactate > 4.0 mmol/L within the last hour)

Analyses

Primary analysis:

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Secondary analysis (will only be made for the modified intention-to-treat population):

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- Shock at time of randomisation Y/N (as defined above) (stratification variable)
- Diabetes at time of randomisation Y/N
- Use of nephrotoxic drugs during current admission and prior to randomisation Y/N
- Previous renal dysfunction ('normal' creatinine >100 µmol/l = baseline variable #2) Y/N
- Acute Kidney Failure at randomisation (renal failure defining severe sepsis as defined above)

Continuous covariate
- Age

Ordinal covariates
- SAPS II
- SOFA-score

Outcomes

Primary outcome measure
The composite outcome measure of 90-day mortality or end-stage kidney disease defined as dialysis-dependency 90 days after randomisation (+/- 4 days) as retrieved from the National Patient Hospital Register and the National Dialysis Database.

These two outcome measures will also be analysed separately.

Secondary outcome measures

- Twenty-eight-day, 6-month and 1-year mortality

- Time to death or censoring

- SOFA-score without cerebral component (see Appendix 5) on day 5 after randomisation.

- Development of kidney failure defined as
  - Renal component of SOFA-score (renal-SOFA) = 3 or higher at any time in the ICU after randomisation, but renal-SOFA < 3 before randomisation (baseline values)

  OR
  - Patient requiring dialysis at any time after randomisation

- Development of kidney failure at any time in the ICU after randomisation defined as
  - Doubling of p-creatinine values = 2 x 'normal creatinine' (baseline variable #2)

- Development of acidosis (pHa < 7,35) in the ICU after randomisation
  - As data from day 1 may reflect baseline characteristics, the analysis will be made with and without day 1.

- Need of dialysis/haemofiltration at any time after randomisation

- Need of ventilation at any time after randomisation

- Days alive without dialysis/haemofiltration in the 90 days after randomisation
  We define the value of a "period of dialysis" which runs from the day where dialysis is initiated till the day where the last dialysis is performed as the number of dates included in the period. The outcome measure is calculated as "1 – (the number of days in the period of dialysis / the number of days alive in the 90 days follow-up period)".

- Days alive without ventilation in 90 days after randomisation
  If the patient is on the ventilator at 8 am, the day is a ventilator-day. The outcome measure is calculated as "1 – (the number of days in ventilator / the number of days alive in the 90 days follow-up period)".

- Days alive and out of hospital in the 90 days after randomisation
  The outcome measure is calculated as "1 – (the number of days in hospital in the 90-day follow-up period / the number of days alive in the 90-day follow-up period).

- Occurrence of severe bleeding in the ICU defined as clinical bleeding needing three units of packed red blood cells or more within 24 hours

- Occurrence of severe allergic reaction in the ICU

Level of statistical significance for all analyses: P = 0.05

Missing Data

Kidney failure at time of randomisation: If the patient doesn't have kidney failure on day 1, this observation will be carried backward.

'Normal' creatinine < 100 mmol/l: If the patient has any creatinine < 100 mmol/l without renal replacement therapy during or after the ICU-stay, the 'normal' creatinine will be considered < 100 mmol/l.

SOFA-score: There will be missing data for patients who

- die before day 5

OR

- are discharged from the ICU before day 5 and are still alive on day 5.

Initially, we will perform a complete case-analysis. Then a supplementary analysis where patients who die before day 5 get the maximum score (20 points), and here patients who are discharged from the ICU and alive on day 5 will get 0 points.

For patients, who are still in the ICU on day 5, last observation will be carried forward.

SAPS-score: Initiallly, we will perform two analyses where 1) missing SAPS-components in group A will be given to the worst possible score AND missing SAPS-components in group B will be given the score zero or 2) missing SAPS-components in group A will be given the score zero AND missing SAPS-components in group B will be given the worst possible score. If there is no difference between these two analyses, we will not impute missing data.

If the frequency of missing data after the above implemented "imputations" is > 5% and the complete case analysis is significant at the 10% value or less, we will perform an additional analysis using the multiple imputation method.

To put significant results into perspective the following sensitivity analysis will be conducted: We define a worst case scenario as one where patients with missing data do not react on the treatment (whatever it may be). Missing data will be imputed according to this scenario. Let P be the estimate of the parameter reflecting the effect of the intervention calculated from the complete case analysis and P-imp be the corresponding estimate calculated from the analysis of the imputed data.
[(P-imp – P)/P-imp]*100% then a ball park figure of the bias is to be expected were the worst case scenario true.
P-imp/ (standard error of P-imp) is calculated and the corresponding p value found to assess the potential impact of this bias on the significance level.

Summary of changes to the Statistical Analysis Plan

At the meeting the 5th March 2012 prior to the beginning of the statistical analyses, the following changes were made:

- The definitions of rate data (ventilator and dialysis) were corrected.

  Dialysis rate = 1 – (the number of days in the period of dialysis / the number of days alive in the 90 days follow-up period)

  Ventilation rate = 1 – (the number of days in ventilator / the number of days alive in the 90 days follow-up period)

- We transformed hospital length of stay to a hospital rate. The reason for this is that a very high number of patients were readmitted to hospital shortly after discharge and still within the 90-day follow-up period. We therefore count the number of days in hospital in the 90-day follow-up period. The outcome measure is calculated as a rate: 1 – (number of days in hospital in the 90-day follow-up period / number of days alive in the 90-day follow-up period).

- Occurrence of severe bleeding and occurrence of severe allergic reaction were added to the analysis plan as these events must be analysed according to the trial protocol.

- Missingness in the SAPS-score will be assessed as follows:
  SAPS-score: Initiallly, we will perform two analyses where 1) missing SAPS-components in group A will be given to the worst possible score AND missing SAPS-components in group B will be given the score zero or 2) missing SAPS-components in group A will be given the score zero AND missing SAPS-components in group B will be given the worst possible score. If there is no difference between these two analyses, we will not impute missing data.