UNIVERSITY OF COPENHAGEN
FACULTY OF HEALTH AND MEDICAL SCIENCES

FLUID RESUSCITATION VOLUMES IN SEPSIS

PhD Thesis

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PREFACE

This thesis is based on three studies performed during my employment at the Department of Intensive Care, Copenhagen University Hospital, Rigshospitalet, from 2013 to 2016.

My time as a researcher during the PhD programme has been filled with unforgettable experiences and I feel fortunate to have been given the opportunity to do such interesting and exciting research with a group of inspirational and cheerful people. There are many people to whom I owe big thanks for the scientific progress I have made and not the least the enjoyable time I have had during these last years.

First and foremost, I am grateful to supervisors Anders Perner, Jørn Wetterslev and Nicolai Haase. I cannot thank you enough for entrusting with a PhD programme that started with blank piece of paper and ended up with the CLASSIC Trial and the present thesis. It has been an absolute privilege to have Anders Perner as my principal supervisor both on the present PhD programme and during my pre-graduate research; always striving for the highest standards and always emphasising that research is a team effort. Anders, I consider you to be my mentor, my colleague, and my friend – had it not been for you, I am unsure that I would have developed my present passion for research and my still increasing fascination of intensive care. Having Jørn Wetterslev as a supervisor has also been a privilege; Jørn, I have thoroughly enjoyed our many discussions on how to achieve the highest methodological standards – it has been inspirational, and your frequent scientific anecdotes have been educational and amusing. Nicolai Haase has offered me his guidance since I was a pre-graduate researcher where Nicolai was a PhD student. Nicolai, your impressive scientific understanding and your relentless helpfulness helped convince me that I wanted to do research and gave me something to strive for as a PhD student.

The CLASSIC trial was a rather complex trial to conduct, and it would not have been feasible to succeed without the dedication and enthusiasm of the CLASSIC trial investigators and clinical staff at each of the nine participating ICUs. I have had the privilege to visit all the hospitals and collaborate with outstanding colleagues around Denmark and Finland. I am grateful to the clinical staff of nurses and doctors at Rigshospitalet who I have worked closely with during the CLASSIC trial and who supported the research despite often busy with the important clinical work. Also, I wish to thank our dedicated team of research nurses Kis Uhre, Jette Degn, Vibeke Knudsen, Vibeke Christiansen, Maj-Brit Kjær, and especially Lizette Nislev who put lots of effort in the conduct of the CLASSIC trial.

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ORIGINAL PAPERS

The present PhD thesis is based on the following papers:

I. **Hjortrup PB**, Haase N, Wetterslev J, Perner A (2016) Associations of hospital and patient characteristics with fluid resuscitation volumes in patients with severe sepsis: Post hoc analyses of data from a multicentre randomised clinical trial. PLoS One 11(5): e0155767.

II. **Hjortrup PB**, Haase N, Bundgaard H, Thomsen SL, Winding R, Pettilä V, Aaen A, Lodahl D, Berthelsen RE, Christensen H, Madsen MB, Winkel P, Wetterslev J, Perner A (2016) Restricting volumes of resuscitation fluid in adults with septic shock after initial management: the CLASSIC randomised, parallel-group, multicentre feasibility trial. Intensive Care Med 42(11):1695-705.

III. **Hjortrup PB**, Haase N, Wetterslev J, Lange T, Bundgaard H, Rasmussen BS, Dey N, Wilkman E, Christensen L, Lodahl D, Bestle M, Perner A. Effects of fluid restriction on measures of circulatory efficacy in adults with septic shock: post hoc analyses of the CLASSIC randomised clinical trial. *Accepted for publication in ACTA Anaesthesiologica Scandinavica – In press.*

SUMMARY

Background

Fluid resuscitation has been a key intervention in the circulatory management of sepsis and septic shock for decades and is promoted by international clinical practice guidelines. However, there is limited data supporting these recommendations and observational and indirect data have suggested harm.

Our primary aim was to assess the feasibility and explore the safety of a protocol aimed at reducing volumes of resuscitation fluids in patients with septic shock who had received the initial fluid management. Additionally, we aimed at assessing variations in the use of resuscitation fluids in clinical practice.

Methods

We conducted the Conservative vs. Liberal Approach to fluid therapy of Septic Shock in Intensive Care (CLASSIC) multicentre randomised clinical feasibility trial in which we randomly assigned intensive care patients with septic shock who had undergone the initial management to either a fluid restriction protocol or a protocol aiming at standard care (Study II). The co-primary outcome measures were cumulated volumes of resuscitation fluids the first five days after randomisation and during the entire intensive care unit (ICU) stay. Exploratory patient-centred outcomes were also assessed.

Additionally, we conducted an observational study of the 6S trial database where we investigated the association between hospital- and patient-characteristics and fluid volumes (Study I) and a post hoc analysis of the CLASSIC trial where we assessed the effect of reducing volumes of resuscitation fluids on measures of circulatory efficacy (Study III).

Results

Of the 153 patients who underwent randomisation in Study II, 151 were included in the intention-to-treat population. The cumulated volumes of resuscitation fluid given in the ICU the first five days following randomisation and during the entire ICU stay were lower in the fluid restriction group compared with the standard care group (mean differences -1.2 L (95% CI -2.0 to -0.4); P<0.001 and -1.4 L (95% CI -2.4 to -0.4); P<0.001, respectively. Total fluid inputs and cumulated fluid balances did not differ with statistical significance. All patient-centred outcome measures favoured fluid restriction and the proportion of patients with worsening of acute kidney injury appeared to be lower in the fluid restriction group (27/73 (37%) vs. 39/72 (54%); P=0.03), but our trial was not powered to show any differences in these outcomes. In the post hoc analysis (Study III), we did not find any indication that restricting fluid resuscitation resulted in worsening of measures of circulatory efficacy.

In the observation analysis of the 6S trial database (Study I), we found differences in volumes of resuscitation fluids between ICUs (P<0.001) which could not be explained by patient characteristics at baseline.

Conclusion

Our randomised clinical trial was the first to successfully randomise patients with septic shock to a protocol specifically aimed at reducing volumes of resuscitation fluids and achieve separation between the two groups. The results of the patient-centred outcome measures added to the growing body of literature indicating harm with higher volumes of resuscitation fluids. Thus, the studies in the present thesis have illustrated feasibility and indicated safety of a protocol reducing volumes of resuscitation fluids, illustrated variations in clinical practice and questioned the circulatory efficacy of fluid resuscitation. We need further large-scale trials with different protocols of higher vs. lower volumes of resuscitation fluid in order to provide high-quality evidence-based fluid therapy to future patients with septic shock.

SUMMARY IN DANISH (DANSK RESUMÉ)

Baggrund

Intravenøs væskebehandling har i årtier været en hjørnesten i kredsløbsbehandlingen af sepsis (blodforgiftning) og anbefales i internationale retningslinjer. Der er dog få data der støtter disser anbefalinger, og observationelle og indirekte data har antydet skadelige effekter.

Vores primære formål var at undersøge gennemførligheden af et forsøg med en protokol, der begrænsede brug af intravenøse væsker ved kredsløbssvigt (resuscitationsvæske), og eksplorativt at vurdere patientsikkerheden af denne protokol. Derudover ønskede vi at undersøge om der er variationer i brugen af resuscitationsvæske i klinisk praksis.

Metoder

Vi gennemførte et multicenter, klinisk forsøg, hvor vi randomiserede intensivpatienter med septisk shock, som havde fået den initiale væskebehandling, til enten en protokol med begrænsninger i brug af resuscitationsvæske eller en protokol tiltænkt at reflektere vanlig praksis (Delprojekt 2). De ko-primære effektmål var kumuleret resuscitationsvæske-volumen givet de første fem dage på intensivafdelingen efter randomisering samt under hele intensivindlæggelsen. Eksplorativt blev også patient-vigtige effektmål undersøgt.

Derudover gennemførte vi et observationelt studie af 6S forsøgsdatabasen (Delprojekt 1), hvor vi undersøgte associationen mellem hospitals- og patientkarakteristika og resuscitationsvæske-volumen og en post hoc analyse af CLASSIC forsøget, hvor vi undersøgte kredsløbsmæssige effekter af at begrænse resuscitationsvæske-volumen (Delprojekt 3).

Resultater

Vi inkluderede 151 af de 153 randomiserede patienter i Delprojekt 2 i analyserne. Det kumulerede resuscitationsvæske-volumen, der blev givet i de første fem dage efter randomisering og under hele intensivindlæggelsen var lavere i væskerestriktions-gruppen sammenlignet med standard praksis-gruppen (gennemsnitlig forskel hhv. -1.2 L (95% CI, -2.0 til -0.4); P<0.001 og -1.4 L (95% CI, -2.4 til -0.4); P<0.001). Der var ikke statistisk signifikant forskel mellem grupperne i totale væskeindgift eller kumulerede væskebalancer. De patient-vigtige effektmål favoriserede væskerestriktionsgruppen og andelen af patienter med forværring af nyresvigt var lavere i væskerestriktionsgruppen (27/73 (37%) vs. 39/72 (54%);P=0.03), men vores forsøg havde ikke statistisk styrke til at vurdere disse effektmål tilstrækkeligt. I post hoc analysen fandt vi ingen antydning af at væskerestriktion forværrede kredsløbsparametre (Delprojekt 3).

I den observationelle analyse af 6S forsøgsdatabasen (Delprojekt 1) fandt vi forskelle i brug af resuscitationsvæske mellem individuelle intensivafdelinger (P<0.001), som ikke kunne forklares ved patient-karakteristika ved baseline.

Konklusion

Vores kliniske forsøg var det første, som randomiserede patienter med septisk shock til en protokol specifikt tiltænkt at reducere resuscitationsvæske-volumen og opnåede separation mellem de to grupper. Analyserne af de patient-vigtige effektmål indikerede, i lighed med den eksisterende litteratur, skadelige effekter af højere resuscitationsvæske-volumen. Derved har studierne i denne afhandling illustreret gennemførlighed og indikeret sikkerhed af en protokol, der reducerer resuscitationsvæsker, illustreret variationer i klinisk praksis samt sat yderligere spørgsmålstegn ved resuscitationsvæskes gavnlige kredsløbsmæssige effekter. Der er således behov for store velgennemførte forsøg der sammenligner forskellige væskevolumen-protokoller for i fremtiden at kunne yde en evidensbaseret væskebehandling af høj kvalitet til gavn for patienter med septisk shock.

BACKGROUND

Sepsis and septic shock

The earliest descriptions of sepsis (from Greek σῆψις: the decomposition of organic matter) date back to the classic antiquity with Hippocrates and Galen. The understanding of sepsis evolved during the following centuries, especially with the development of germ theory by the work of Pasteur, Lister and Semmelweis in the middle of the 19th century. It was not until late in the 20th century it was proposed that the host response to a microorganism, rather than the microorganism itself, was the most important mechanism behind the sepsis syndrome.[1] In 1992, an international consensus committee defined sepsis as a systemic inflammatory response to an infection and introduced the systemic inflammatory response syndrome (SIRS).[2] Septic shock was defined as sepsis with hypotension despite fluid resuscitation along with the presence of perfusion abnormalities.[2] In 2016 – after the planning and conduction of the studies in the present thesis – a third international consensus definition of sepsis and septic shock was published (Sepsis-3).[3] Sepsis was then defined as a life-threatening organ dysfunction (≥2 change in SOFA[4] score caused by a dysregulated host response to an infection), and septic shock was defined as sepsis with persisting hypotension with need of vasopressors and a plasma lactate above 2 mM despite 'adequate fluid resuscitation'; of note, the task force could not provide a definition of what constituted adequate fluid resuscitation.[3]

Regardless of the exact definition, sepsis is characterised by a systemic inflammation in response to an invading pathogen. The excessive inflammatory response may affect the endothelium and the coagulation system as well as induce tissue/organ injury, eventually leading to organ dysfunction.[5,6] The cardiovascular characteristics of sepsis include vasodilatation[7] which may lead to hypotension, myocardial depression[8] which may lead to right and/or left ventricular failure, and increased vascular permeability[9] which may lead to increased capillary leakage and tissue oedema.

Despite advances in critical care, the risk of death with sepsis remains high; mortality rates range from 20-50% in severe sepsis and septic shock depending on the definition and case-mix.[10,11] In the US, the incidence of severe sepsis was 3 per 1,000 population in 2007 and the estimated costs were $24.3 billion.[12] This would make sepsis one of the leading causes of death and burdens to patients and society.

Intravenous fluid therapy

In 1832, the second cholera pandemic reached the United Kingdom. Cholera was characterised by profuse watery diarrhoea and vomiting. The predominant choices of treatment at that time were venesection, emetics, and laxatives.[13] Inspired by ideas of William O'Shaugnessy,[14] Thomas Latta boldly tried treating patients with cholera with 'copious injection of aqueous and saline fluids into the veins.'[15] The results were astounding, and the patients were relieved of their symptoms, including alleviation of a burning thirst. In one of the cases he described: '… within forty-eight hours she smoked her pipe free from distemper'.[15] Thus, the first documented treatment with intravenous (IV) fluids was replenishment of fluid losses. Since then, the use of IV fluids has expanded - especially in critical illness; IV fluids may be administered as means

of delivering medicinal drugs and nutrition, correcting electrolyte disturbances, maintaining fluid balance, and administered with the specific aim of improving the circulation.

Fluid resuscitation in sepsis

Historic perspective

The work by Latta illustrated the importance of replacing fluids in case of loss of large amounts of fluids. In 1914, the physiologists Sydney Patterson and Ernest Starling investigated the relationship between the venous pressure and the cardiac output (**Figure 1**).[16] Along other experiments by contemporary physiologists, these findings led to what we now call 'Starling's Law of the Heart' or the 'Frank-Starling Mechanism' which states that when all other variables are constant, a larger end-diastolic volume increases the stroke volume until a certain point where the heart becomes over-distended and the stroke volume decreases.

The Frank-Starling Mechanism provided physiological rationale for fluid administration beyond the replacement of losses, i.e. if fluid administration increased the end-diastolic volume, the stroke volume was in turn likely to increase. Fluid administration aiming at improving the circulation in shock states was promoted in the 1960s by one of the pioneers in critical care medicine, Max H. Weil. He and colleagues proposed to measure the central venous pressure (CVP) using a central venous catheter in the superior vena cava or right atrium in order to assess right ventricular function.[17] Ten years later, Weil and Shubin specifically recommended using the CVP to guide fluid resuscitation in shock caused by bacterial infections by stating: 'If the central venous pressure is not in excess of 16 cm of water [≈12 mmHg], fluid therapy is best attempted'.[18]

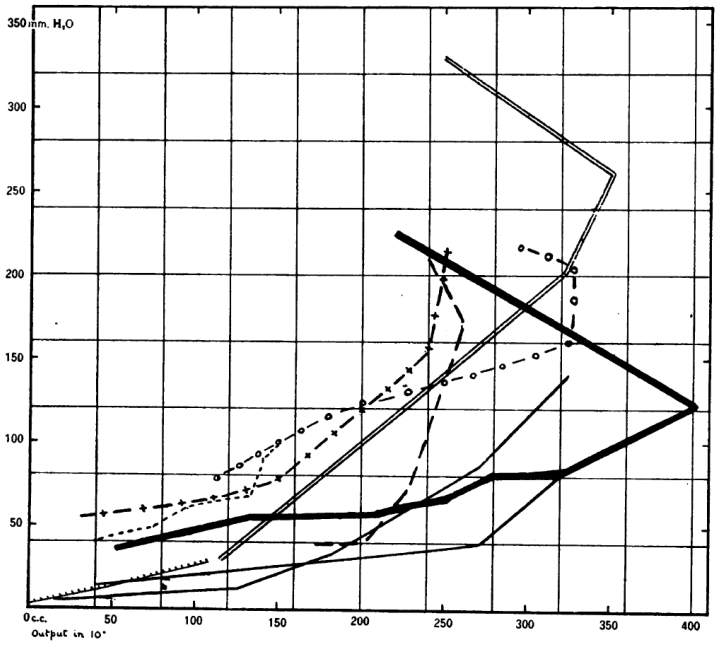

Figure 1. Relationships between the venous pressure (ordinate) and the output (abscissa) in nine canine experiments. Reprinted from Patterson and Starling[16] with permission from John Wiley and Sons (license no: 3950750366270)

Early fluid resuscitation in septic shock

In 2001, the landmark randomised trial on Early Goal-Directed Therapy (EGDT) in the first 6 hours of septic shock by Rivers and colleagues reported a remarkable reduction in mortality with an EGDT protocol compared with a standard care protocol.[19] The trial was a single-centre trial performed in an emergency department in Detroit, Michigan where 263 adult patients with septic shock were randomised. In both the EGDT and the standard care protocol, fluid administration was to be guided by the central venous pressure with a target of 8-12 mmHg and the protocols mainly differed by the use of central venous oxygen saturation ($ScvO_2$) and hematocrit to guide dobutamine and blood transfusions in the EGDT group; nevertheless, patients randomised to the EGDT protocol received more fluids in the 6 hour intervention period compared with patients randomised to standard care (mean 5.0 L vs. 3.5 L, $p< 0.001$).[19] The EGDT protocol was still recommended in the 2012 Surviving Sepsis Campaign (SSC) guidelines;[20] the use of lactate clearance to guide interventions in early septic shock was also recommended as a viable option based on the results of two RCTs comparing conventional EGDT with lactate clearance modified EGDT protocols.[21,22] Three high quality large-scale multicentre trials - the PROCESS trial,[23] the ARISE trial,[24] and the PROMISE trial[25] - and an updated systematic review with meta-analysis did not find a beneficial effect of EGDT in early septic shock.[26] Also, the use of CVP to guide fluid resuscitation has been questioned by systematic reviews with meta-analyses.[27,28] On the other hand, adherence to the SSC resuscitation bundles has been associated with decreased mortality in two large cohort studies.[29,30] In the 2016 SSC guidelines, the 6-hour bundle with the EGDT protocol had been omitted.[31]

Fluid resuscitation beyond the initial management

The balance between benefit and harm with fluid resuscitation is complex and have not been fully elucidated; a too restrictive approach to fluid therapy carries the risk of hypoperfusion and organ impairment due to low cardiac output, and a too liberal approach carries the risk of organ impairment due to peripheral/organ oedema including pulmonary oedema, and the risk of electrolyte disturbances. Additionally, damage to the endothelial glycocalyx in response to hypervolemia has been proposed.[32] In the 2012 SSC clinical practice guideline, it was recommended to continue to administer fluids as long as the circulation improves either based on dynamic (e.g. stroke volume variation) or static (e.g. arterial blood pressure) variables; this recommendation was ungraded due to lack of firm evidence.[20]

Observational studies have indicated harm with increasing fluid balance and fluid input. In a retrospective analysis of the VASST trial, increased cumulative fluid balances at 12 hours and at 4 days were associated with increased mortality in adult patients with septic shock in adjusted analyses; higher fluid balance was mainly driven by increased fluid input.[33] Similarly, cumulative fluid balance at 72 hours in sepsis was associated with increased mortality in a multivariate analysis of the SOAP study cohort[34] and increased daily fluid balances from day 2 until day 7 have been associated with increased mortality in septic shock in adjusted analyses.[35] However, observational studies on fluid volumes are inherently prone to bias (time-dependency, confounding by indication, and competing risks) which hampers inference about causality.

Indirect evidence – i.e. evidence not directly related to fluid volumes in adult septic shock – has also suggested harm. The FEAST randomised clinical trial, conducted in a resource limited setting, where 3,141 febrile children with impaired circulation were randomised to either a saline bolus, an albumin bolus, or no bolus reported increased mortality at 48 hours in both bolus groups as compared with the no bolus group and was stopped early.[36] The FACTT trial randomised 1,000 patients with acute lung injury to conservative or liberal fluid management strategies; the protocols were complex and included fluid input, furosemide and dobutamine.[37] There was no statistically significant between-group difference in the primary outcome of mortality, but increased number of ventilator-free days in the conservative group.

Thus, fluid resuscitation has been a mainstay intervention in the hemodynamic management of septic shock for decades and has been promoted by international clinical practice guidelines, but there are limited high-quality data to support these recommendations and potential harm has been suggested.

AIM OF STUDIES

Our primary aim was to develop a protocol restricting resuscitation fluids in adults with septic shock and to assess the effect of this protocol in reducing volumes of resuscitation fluid and explore on patient-centred outcomes as compared with a standard care protocol across multiple centres. Secondarily, we aimed at identifying characteristics associated with differences in fluid volumes in patients with severe sepsis.

STUDY OUTLINE

The present PhD thesis comprises three studies:

Study I: An observational analysis of the 6S trial database assessing whether hospital and patient baseline characteristics were associated with differences in fluid resuscitation volumes given in the first 3 days of the study.

Study II: The Conservative vs. Liberal Approach to fluid therapy of Septic Shock in Intensive Care (CLASSIC) randomised clinical feasibility trial assessing the effects of a protocol restricting volumes of resuscitation fluids vs. a protocol aiming at standard care, primarily on fluid volumes and exploratory on patient-centred outcomes.

Study III: A post hoc analysis of the CLASSIC trial assessing the effects of restricting volumes of fluid resuscitation on measures of circulatory efficacy.

STUDY I: ASSOCIATIONS OF HOSPITAL AND PATIENT CHARACTERISTICS WITH FLUID RESUSCITATION VOLUMES IN PATIENTS WITH SEVERE SEPSIS: POST HOC ANALYSES OF DATA FROM A MULTICENTRE RANDOMISED CLINICAL TRIAL

Methods

Overview and design

A retrospective observational study using data from the Scandinavian Starch for Severe Sepsis / Septic Shock (6S) trial database.[38]

The 6S trial

In brief, the 6S trial randomised adult patients with severe sepsis and need of fluid resuscitation to treatment with hydroxyethyl starch (HES) 130/0.42 (Tetraspan, B Braun Medical AG, Melsungen, Germany) or Ringer's acetate (Sterofundin, B Braun Medical AG) between 2009 and 2011 in 26 intensive care units (ICUs) in Denmark, Norway, Finland and Iceland. The participating ICUs not located in Denmark were all university hospitals. Exclusion criteria included use of renal replacement therapy, severe hyperkalaemia (plasma potassium above 6 mM), acute burn injury > 10% of body surface area, and treatment with >1,000 ml of synthetic colloid in the 24 hours prior to assessment for eligibility.

If the maximum dose of 33 ml/kg/24h masked trial fluid was exceeded, open label trial fluid (Ringer's acetate) was administered. Patients were withdrawn from the trial intervention in case of commencement of renal replacement therapy for AKI, bleeding, or allergic reactions.

A detailed protocol of the 6S trial and the outcomes have previously been published.[38,39]

Patients

We included patients randomised in the 6S trial for whom fluid data from 24 hours before randomisation until day 3 post-randomisation were available. Thus, patients who had died or had been discharged within the first 3 days after randomisation, and patients with missing fluid data were not included.

Outcome measures

The primary outcome measure, volume of resuscitation fluid, was defined as all masked and open-label trial fluids given in the first 3 days after randomisation combined with crystalloids (not including fluids with medication) and colloids given 24 hours before randomisation (day 0). If a patient was withdrawn from the trial intervention, then all crystalloids (not including fluids with medication) and colloids given from the time of withdrawal until the end of day 3 after randomisation were regarded as fluid resuscitation.

The secondary outcome measure was total fluid input from day 0 until the end of day 3.

Explanatory variables

We assessed associations between hospital characteristics and fluid volumes with the following variables: Danish hospital (yes/no), university hospital (yes/no), and individual hospitals with at least 25 patients randomised in the 6S trial in the models.

We decided *a priori* to include the following patient baseline characteristics in the multivariate analyses: Simplified Acute Physiology Score (SAPS II)[40], age, weight, highest plasma lactate at randomisation, surgery performed prior to randomisation, intervention group and individual Sequential Organ Failure Assessment (SOFA) subscores (cardiovascular, renal, liver, coagulation and respiratory).[4]

Statistical analysis

We performed the analyses using general linear models with fluid volumes as outcome. To assess hospital characteristics, we performed multiple linear regression analyses adjusted for patient characteristics. To assess patient characteristics, we performed multivariate linear regression adjusted for trial site.

If > 5% of baseline values were missing, multiple imputations of the missing values were performed in an attempt to reduce the risk of bias.[41] Complete case and sensitivity analyses were also performed. We assessed the patterns of residuals for each analysis to ensure adequate goodness of fit.

Results

Of the trial cohort of 798 patients, we included the 654 (82%) patients who had fluid volumes registered from day 0 to 3 (65 (8%) had died, 75 (9%) had been discharged, and 4 (<1%) had missing fluid data).

Fluid volumes

The median volume of resuscitation fluid administered from day 0 to day 3 was 7,300 ml (interquartile range (IQR) 5,000-10,000 ml) and the median total fluid input was 16,912 ml (IQR 13,681-20,513 ml). Fluids given with medication and nutrition combined comprised 72% of the difference between fluid resuscitation volume and total fluid input.

Hospital characteristics and fluid volumes

Adjusted for patient baseline characteristics, the volumes of resuscitation fluid and total fluid inputs differed with statistical significance between individual trial sites (**Figure 2**). The estimated difference in fluid volumes adjusted for baseline characteristics for Danish vs. non-Danish hospitals was -641 ml (95% CI -1,787 to 506; p=0.27) for resuscitation fluid and 1,773 ml (314 to 3,232; p=0.02) for total input. For university vs. non-university hospitals, the estimated difference was -825 ml (-1,500 to -150; p=0.02) for resuscitation fluid and -618 ml (-1,604 to 368; p=0.22) for total fluid input.

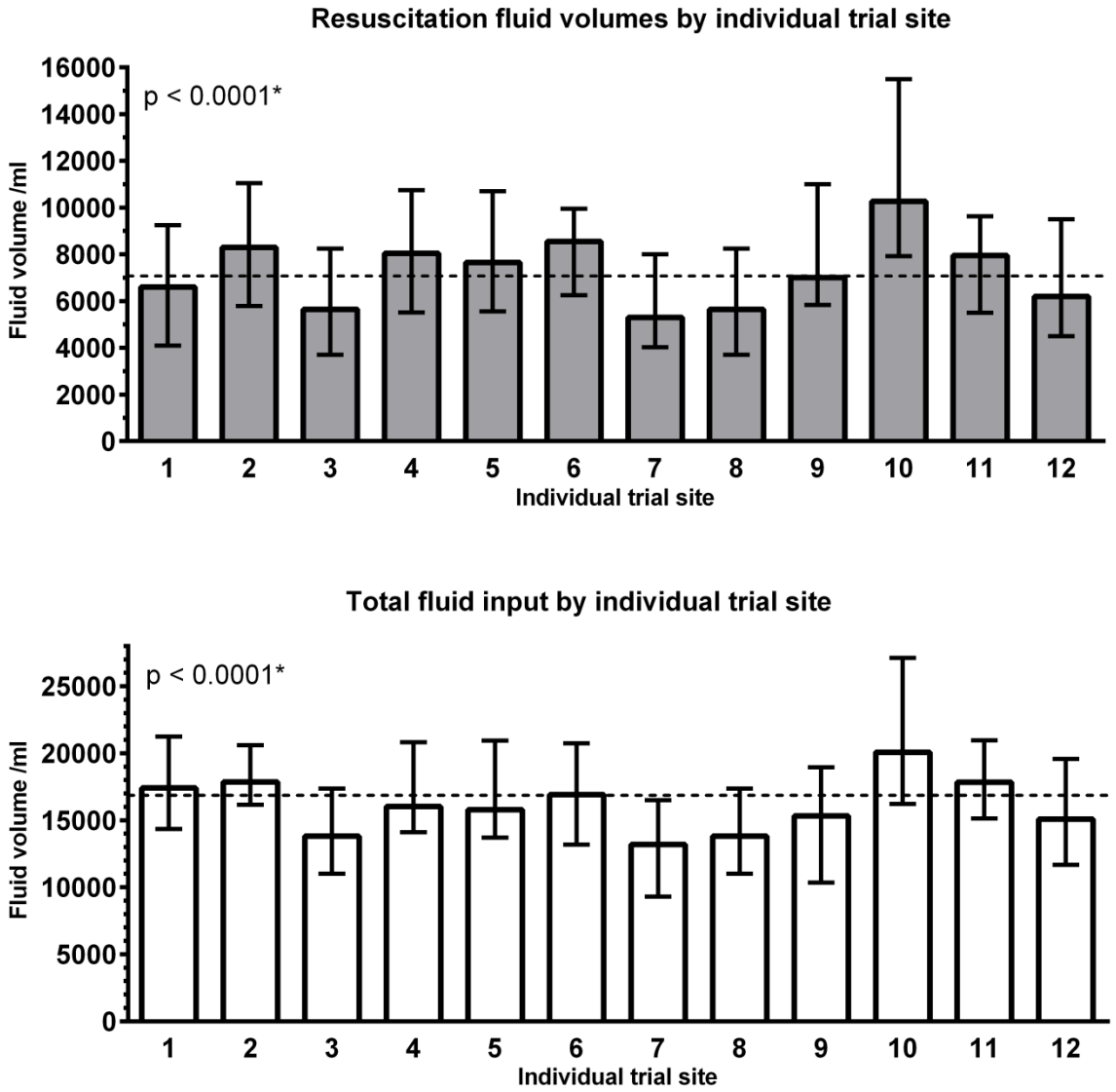

Figure 2. Resuscitation fluid volume (top panel) and total fluid input (bottom panel) by individual trial site with at least 25 randomised patients (n = 542). Fluid volumes presented as median with interquartile range (error bars). The horizontal dashed lines denote medians for all patients. * P-value for trial site in a generalised linear model. (presented in paper I)

Patient characteristics and fluid volumes

In the multivariate generalised linear model, increased lactate, higher cardiovascular and renal SOFA subscores, lower respiratory SOFA subscore and having surgery performed were associated with higher

volume of resuscitation fluid (**Table 1)**. In addition, patient weight at baseline and higher coagulation SOFA subscore were associated with increased total fluid input.

Table 1. Patient baseline characteristics and fluid volumes given from day 0 to day 3 (presented in paper I)

	Resuscitation fluids (95% CI) (ml)	P-value*	Total fluid input (95% CI) (ml)	P-value*
Age per year	-7 (-34 – 20)	0.60	-24 (-62 – 14)	0.21
SAPS II per unit	11 (-13 – 36)	0.35	14 (-24 – 51)	0.48
Weight per kg	15 (-0.3 – 31)	0.055	41 (18 – 64)	0.0005
Highest lactate per mmol/l	193 (53 – 332)	0.0069	289 (77 – 502)	0.008
Surgery vs. No surgery	1376 (732– 2019)	< 0.0001	2941 (2006 – 3877)	< 0.0001
HES vs. Ringer's	-276 (-844 – 292)	0.34	305 (-518 – 1129)	0.47
SOFA subscores per unit				
- Cardiovascular subscore	375 (184 – 566)	0.0001	632 (340 – 924)	< 0.0001
- Renal subscore	269 (11– 528)	0.04	353 (8 – 698)	0.045
- Coagulation subscore	63 (-232 – 357)	0.68	885 (454 – 1315)	< 0.0001
- Liver subscore	-43 (-460 – 374)	0.84	61 (-541 – 663)	0.84
- Respiratory subscore	-397 (-729 – -65)	0.02	-215 (-676 – 245)	0.36

Multivariate linear regression analysis adjusted by trial site. * P-values for type III sum of squares. Individual SOFA subscores range from 0-4 with 4 being the most severe score. CNS component of the SOFA score was not reported in the 6S trial and is not included in the analysis. Abbreviations: CI, confidence interval. HES, hydroxyethyl starch. ICU, intensive care unit. SOFA, Sequential Organ Failure Assessment.

Analyses challenging the robustness of the results

Since 25% of the patient had missing SAPS II at baseline, we performed the primary analyses using a multiple imputations technique; complete case analyses did not affect the results noticeably.

Restricting the primary outcome measure to only including fluid resuscitation administered after randomisation inferred that only individual trial site, highest lactate, and renal SOFA subscore were associated with differences in volumes of resuscitation fluid.

When analysing individual trial sites as a random-effects variable, individual trial site remained significantly associated with differences in fluid resuscitation volumes (p=0.03).

Conclusions

We observed differences in the administered volumes of resuscitation fluid between individual hospitals which could not be explained by patient characteristics which emphasized the need for RCTs on volumes of resuscitation fluid in patients with severe sepsis.

STUDY II: RESTRICTING VOLUMES OF RESUSCITATION FLUID IN ADULTS WITH SEPTIC SHOCK AFTER INITIAL MANAGEMENT: THE CLASSIC RANDOMISED, PARALLEL-GROUP, MULTICENTRE FEASIBILITY TRIAL

Methods

Overview and design

The Conservative vs. Liberal Approach to fluid therapy of Septic Shock in Intensive Care (CLASSIC) trial was an investigator-initiated multicentre, randomised, parallel-group, feasibility clinical trial where patients were randomised from September 7th 2014 to August 7th 2015 in 9 ICUs in Denmark and Finland. Randomisation was web-based, and allocation was concealed using permuted blocks of varying sizes of 2 and 4 with stratification according to trial site. The trial was approved by the Ethics Committees in Denmark (H-3-2014-057) and Finland (233/13/03/02/2014) and by the Danish Health and Medicines Agency (2014043517) and the Danish Data Protection Agency (30-1265). The trial was registered at clinicaltrials.gov (NCT02079402). The statistical analysis plan was written before the end of the trial.

Patients

Adult patients in the ICU with septic shock with severe circulatory impairment for no more than 12 hours who had received the initial fluid resuscitation were eligible for randomisation. Exclusion criteria included any form of renal replacement therapy (RRT), severe hyperkalaemia, plasma creatinine > 350 µmol/l, life-threatening bleeding mechanical ventilation with FiO_2 > 0.80 and positive end-expiratory pressure (PEEP) > 10 cmH_2O, and a decision not to give full life support.

Interventions

Patients were assigned 1:1 to either a protocol restricting fluid resuscitation or a protocol aimed at standard care. For all patients, a mean arterial pressure (MAP) of at least 65 mmHg (or a target decided by the clinicians) was maintained by the use of continuous infusion of noradrenaline. The choice of type of crystalloid was at the discretion of clinicians; use of colloids for resuscitation was regarded as a protocol violation in both groups. Substitution of overt fluid losses was allowed in both groups.

In the fluid restriction group, isotonic crystalloid (saline or Ringer's solutions) fluid boluses of 250-500 mL could be given in the ICU (but was not mandated) only in case of severe hypoperfusion defined as either (1) plasma concentration of lactate of $\geq$ 4 mM, (2) MAP below 50 mmHg, (3) mottling beyond the edge of the kneecap (mottling score >2)[42], or (4) severe oliguria in the first 2 hours after randomisation.

In the standard care group, crystalloid (saline or Ringer's solutions) fluid boluses could be given in the ICU as long as hemodynamic variables improved; the choice of variable(s) was at the discretion of the treating clinicians.

Outcomes

We primarily assessed volumes of fluid resuscitation defined as volumes of 0.9% saline, buffered crystalloid solutions, and colloid solutions administered in the ICU for circulatory impairment. The two co-primary outcome measures were cumulated volumes of resuscitation fluid administered in the first 5 days after randomisation and during the entire ICU stay, respectively.

Secondary outcome measures were total fluid inputs, cumulated fluid balances, number of patients with major protocol violations in the fluid restriction group, and rates of serious adverse reactions in the ICU to both isotonic crystalloids and noradrenaline.

Patient-centred exploratory outcomes included mortality, number of patients with ischaemic events in the ICU, renal outcomes, and use of mechanical ventilation.

Statistical analyses

The statistician performed all the analyses blinded for the intervention and according to the statistical analysis plan which was defined prior to accessing the database. We performed the analyses in the intention-to-treat population defined as all randomised patients except those who withdrew consent for the use of data. In the predefined primary analyses, we compared the two groups by the non-parametric van Elteren test or the general linear model for rate data adjusted for the stratification variable (trial site), logistic regression analysis for binary outcome measures adjusted for site and by log-rank test and Cox analysis (adjusted for site) for time to death.

Results

Of 203 patients evaluated for inclusion, 153 underwent randomisation and 151 patients were included in the intention-to-treat analyses (**Figure 3**).

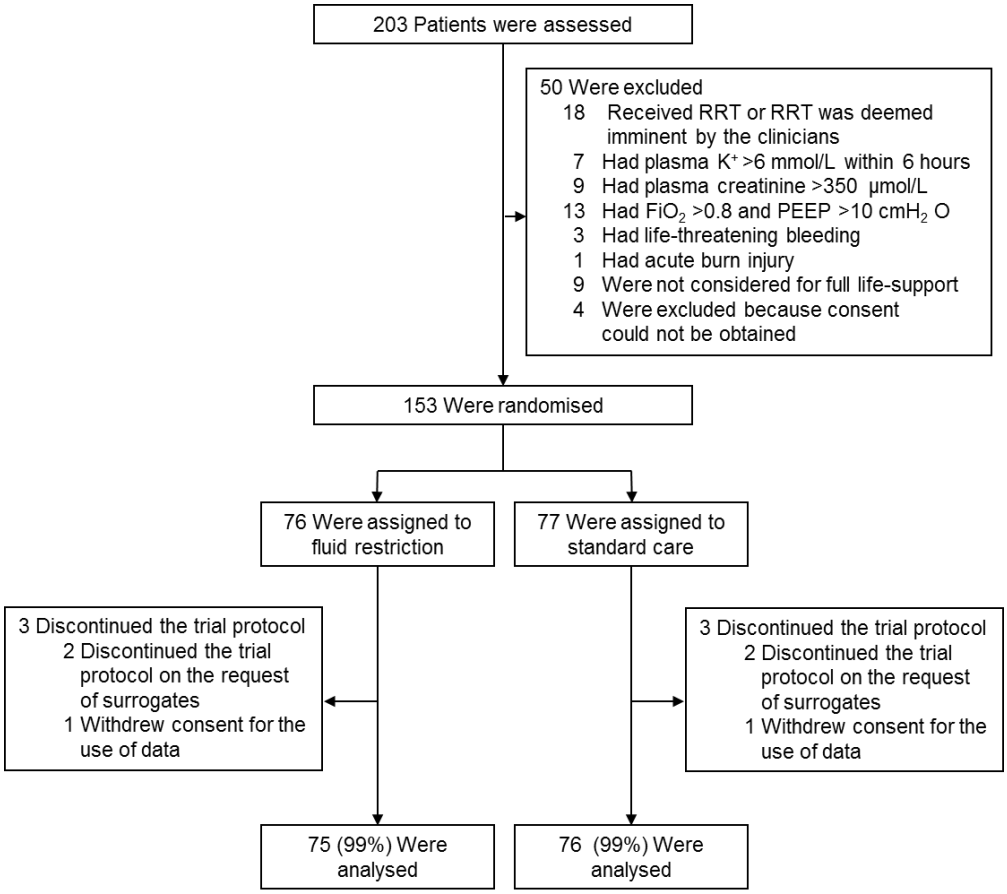

Figure 3. Flow of trial participants in the CLASSIC trial (presented in paper II).

Fluid protocol

In the fluid restriction group, 55 of 75 patients (73%) received fluid resuscitation during a total of 286 episodes vs. 70 of 76 patients (92%) during 464 episodes in the standard care group.

Co-primary outcome measures

The cumulated resuscitation fluid volumes given in the ICU first 5 days after randomisation and during the entire ICU stay were lower in the fluid restriction group compared with the standard care group (mean differences -1.2 L (95% CI, -2.0 to -0.4); P<0.001 and -1.4 L (95% CI, -2.4 to -0.4); P<0.001, respectively (**Figure 4 and Table 2**)).

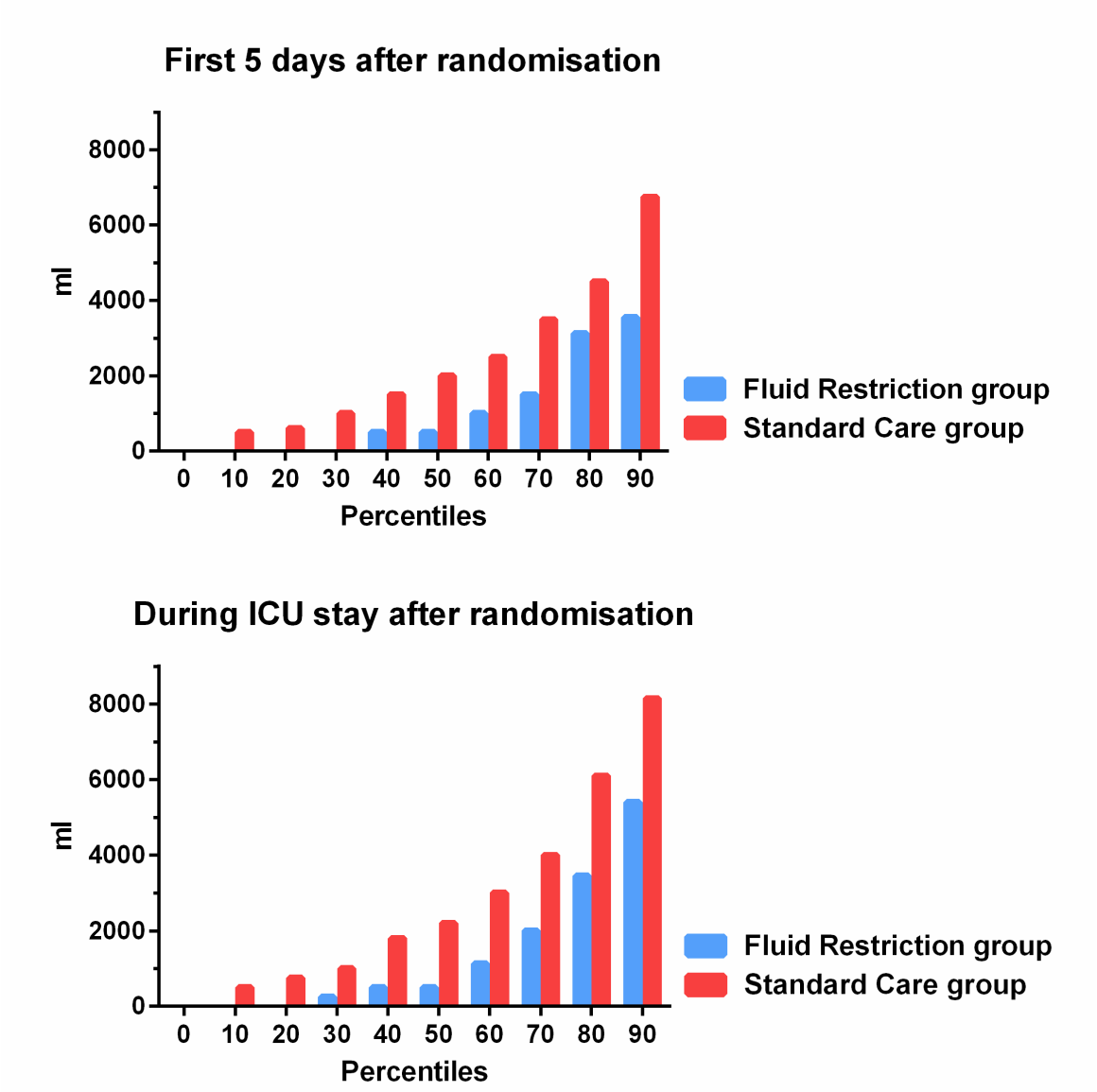

Figure 4. Percentiles of volumes of resuscitations fluids given after randomisation (presented in paper II)

Secondary outcome measures

Total fluid inputs and cumulated fluid balances at day 5 after randomisation and during the entire ICU stay, and rate of SARs did not differ with statistical significance between groups (**Table 2**). In the fluid restriction group, 27 of the 75 (37%) patients had a total of 80 major protocol violations.

Table 2. Primary and secondary outcome measures (presented in paper II)

Outcome	Fluid restriction group (n=75)	Standard care group (n=76)	Fluid restriction vs. standard care (95% CI)	P value
Co-primary outcome measures				
Volumes of resuscitation fluid (mL)				
First 5 days after randomisation	500 (0-2,500) [1,687]	2,000 (1,000-4,100) [2,928]	-1,241 (-2,043 to -439)	<0.001
During ICU stay after randomisation	500 (0-3,250) [1,992]	2,200 (1,000-4,750) [3,399]	-1,407 (-2,358 to -456)	<0.001
Secondary outcome measures				
Total fluid input (mL)[c]				
First 5 days after randomisation	12,411 (5,518-17,035) [11,777]	13,687 (7,163-17,082) [12,597]	-820 (-2,968 to 1,329)	0.45
During ICU stay after randomisation	18,291 (5,518-34,045) [21,459]	16,970 (7,163-29,889) [23,495]	-2,036 (-10,920 to 6,848)	0.65
Cumulated fluid balance (mL)				
First 5 days after randomisation	1,752 (-1,153-3,758) [2,141]	2,680 (407-5,114) [3,289]	-1,148 (-2,531 to 235)	0.06
During ICU stay after randomisation	1,923 (-1,964-5,415) [2,032]	2,014 (-168-4,678) [2,507]	-475 (-2,254 to 1,304)	0.60
Serious adverse reactions				
Number of reactions per day during the ICU stay	0.14 (0-0.50) [0.37]	0.15 (0-0.52) [0.33]	NA	0.85

Values in the two intervention groups are presented as medians (interquartile ranges) [mean].

Abbreviations: CI, confidence interval; NA, not applicable.

Exploratory outcome measures

We observed no statistically significant difference between the two groups for death at day 90, time to death at latest follow-up, number of patients with ischaemic events in ICU (**Figure 5**), days alive without mechanical ventilation (mean 79 vs. 72%, P=0.48) or renal replacement therapy (92 vs. 92%, P=0.70) in the 90-day follow-up period or maximum changes in plasma creatinine in the ICU (median 9 (IQR -13 - 47) vs. 15 (-4 - 62) µmol/L, P=0.36). The number of patients with worsening of acute kidney injury was lower in the fluid restriction group than in the standard care group (**Figure 5**).

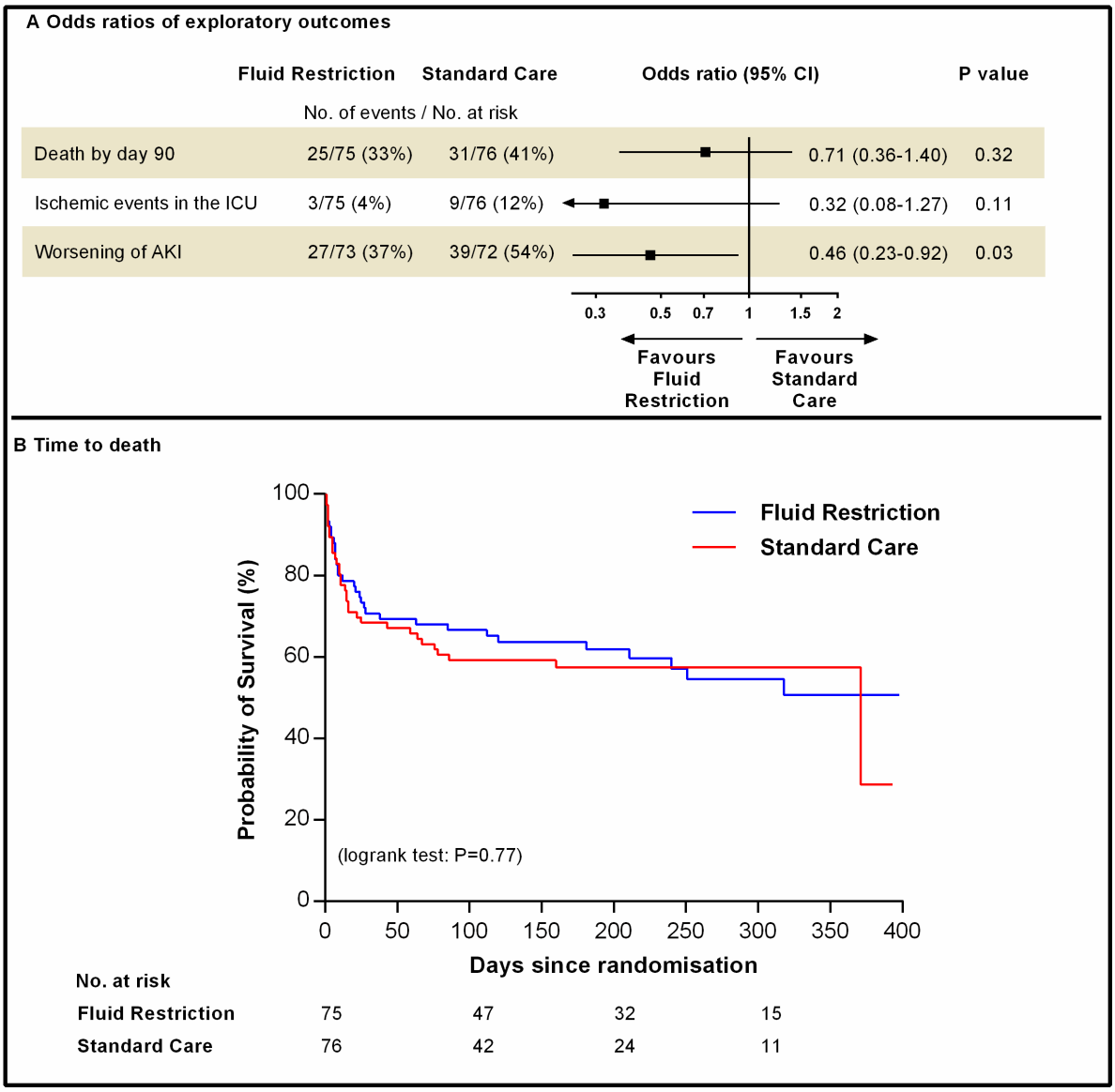

Figure 5. Exploratory outcome measures (presented in paper II)

Conclusions

We observed that ICU patients with septic shock randomised to a protocol aiming at restricting resuscitation fluid received lower volumes of resuscitation fluid as compared with patients randomised to a protocol aiming at standard care. The exploratory outcomes suggested benefit from fluid restriction, but large high-quality trials assessing benefits vs. harms of lower vs. higher volumes of resuscitation fluid are urgently needed to adequately assess these outcomes.

STUDY III: EFFECTS OF FLUID RESTRICTION ON MEASURES OF CIRCULATORY EFFICACY IN ADULTS WITH SEPTIC SHOCK: POST HOC ANALYSES OF THE CLASSIC RANDOMISED CLINICAL TRIAL

Methods

Overview and design

A post hoc analysis of data from Study II. The analyses were defined prior to accessing the variables in question in the database, but after the results of the CLASSIC trial were known. All randomised patients were eligible for the present analyses.

Collection of data

The highest plasma lactate measurements, highest doses of noradrenaline, and the cumulated urinary outputs were recorded in the CLASSIC trial in five time frames in the first 24 hours after randomisation (0 to 3 hours, 3 to 6 hours, 6 to 9 hours, 9 to 12 hours and 12 to 24 hours).

Statistics

The primary analyses of the intervention effect was the effect of restricting fluid resuscitation on changes in the plasma lactate, urinary outputs, and doses of noradrenaline during the first 24 hours after randomisation. We used multiple linear regression with repeated measures adjusted for the stratification variable trial site, and baseline lactate and noradrenaline values as fixed effects and accommodated intra-patient dependencies were accommodated by including random slope and intercept for each patient.

Results

Fluid resuscitation in the first 24 hours after randomisation

Fluid resuscitation was administered to 43 (57%) patients in fluid restriction group vs. 66 (87%) in the standard care group during the first 24 hours. The cumulated fluid resuscitation volume in the first 24 hours after randomisation was median 500 ml (IQR 0-1,500) and 1,250 ml (500-2,500) in the fluid restriction group and standard care group, respectively.

Intervention effect on measures of circulatory efficacy

Values of plasma lactate concentrations (estimated difference in the fluid restriction group vs. the standard care group 0.1 mM; 95% CI -0.7 to 0.9, p=0.86) , doses of noradrenaline (0.01 µg/kg/min; 95% CI -0.02 to 0.05, p=0.48), and urinary outputs (-0.1 ml/kg/hour; 95% CI -0.3 to 0.2, p=0.70) during the first 24 hours after randomisation did not differ with statistical significance between the two intervention groups (**Figure 6)**.

Conclusion

In this post hoc analysis, we did not observe any indications that being randomised to a protocol restricting fluid resuscitation resulted in worsening of plasma lactate levels, doses of noradrenaline, and urinary output during the first 24 hours after randomisation in adults with septic shock randomised to a protocol restricting fluid resuscitation as compared with a protocol aiming at standard care. Despite fluid resuscitation being one of the most frequent interventions in sepsis, efficacy and safety remain to be fully elucidated.

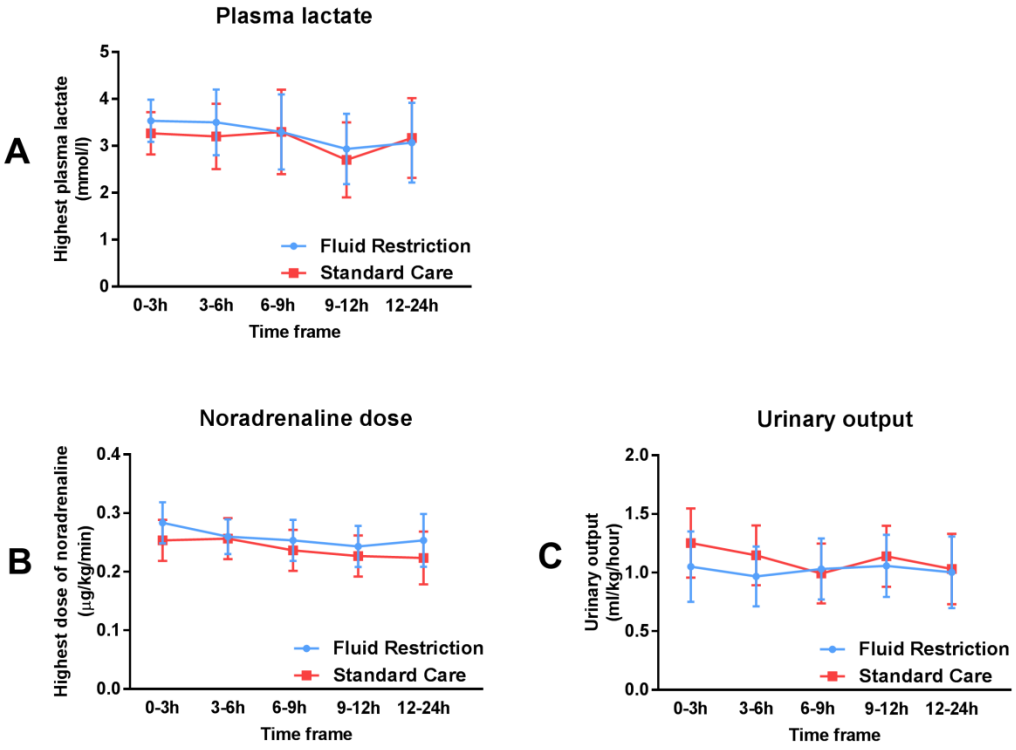

Figure 6. Hemodynamic variables in patients with septic shock in 5 time frames during the first 24 hours after randomisation. (presented in paper III)

DISCUSSION

Principal findings

In the observational analysis of the 6S trial (Study I), we observed between-ICU differences in fluid resuscitation volumes the first 3 days after randomisation which were not explained by patient characteristics at baseline. The indication that the site of admission influenced on fluid volume administration after adjusting for patient characteristics emphasised the need for RCTs on fluid resuscitation volumes in patients with septic shock.

In the CLASSIC trial (Study II), we showed that a fluid restriction protocol was feasible and observed reduced volumes of resuscitation fluids administered in the ICU as compared with a protocol aiming at standard care in patients with septic shock who had undergone initial management. The patient-centred outcomes suggested benefit with fluid restriction including lower rate of worsening of AKI, but the trial was not powered to adequately assess these outcomes.

The post hoc analysis of the CLASSIC trial (Study III) was not predefined and, thus, any conclusions must be drawn with caution. We observed no indication that allocation to the fluid restriction group resulted in impairment of measures of circulatory efficacy in the first 24 hours after randomisation as compared with allocation to the standard care group.

Limitations and strengths – the observational analysis of the 6S trial (Study I)

The main strength of our observational analysis of the 6S trial was the detailed fluid registration in the 6S database which enabled us to exclude much of the IV-fluids administered on other indications than for improving the circulation. Also, the generalisability was likely to be high since the trial was conducted in 26 ICUs in four countries. Importantly, although the 6S trial was a pragmatic RCT performed to the highest standards, a large proportion of the strengths of the 6S trial were restricted to the randomised comparison. We performed observational analyses of the trial database and, thus, the inherent limitations with observational studies did not elude us.

We defined the variables in the models *a priori* which increased the validity of the findings. We chose this approach over a stepwise regression for mainly two reasons. First, we aimed at assessing the association between fluid volumes and all the chosen variables. Second, an approach with stepwise approach increases the risk of over-fitting and may underestimate the contribution of combinations of variables.[43]

We restricted our analyses to patients who were in the ICU 3 days after randomisation; thereby, patients who had died or had been discharged early were excluded. This choice reduced the generalisability to all patients being admitted to the ICU fulfilling the criteria for inclusion in the 6S trial, because it is not known whether a patient will stay in the ICU for at least 3 days upon admission. On the other hand, it reduced the risk of competing risk bias – i.e. patients were not at risk of fluid resuscitation if they had died or been discharged early.

Limitations and strengths - The CLASSIC trial (Study II)

Feasibility trial design

A feasibility trial, also referred to as a pilot trial, is a trial aiming at assessing the feasibility of methods and procedures to inform a subsequent trial on a large scale.[44] Our main reason for designing CLASSIC as a feasibility trial was that we were not sufficiently confident that the protocol would be accepted by all clinical staff across multiple trial sites, and that separation in fluid resuscitation volumes would occur between the two groups. There were two main challenges to the feasibility of the trial. First, if the restrictive protocol was similar to usual care, separation between the two groups would be unlikely even in the case of impeccable protocol adherence. Second, we anticipated a risk of non-adherence to the protocol. Fluid resuscitation in sepsis is a matter of intense discussion – both academically, but in our experience also clinically where differences in opinion are frequent and plenty. Also, the intervention period was round the clock for the entire ICU stay. As a consequence, many clinicians cared for the trial patients, and if just one of these at one point during the trial deemed the protocol unsuitable, the protocol could be violated. The acceptability of the trial intervention has been described as a valid objective for a feasibility trial, although most often described as acceptability from the patients' perspective and not the clinicians'.[45] Another recent pilot trial randomised 82 patients with septic shock to either targeted fluid minimization or usual care, but did not achieve statistically significant differences in the primary outcome measures of colloid, crystalloid and other continuous fluid infusions.[46]

The multicentre setting was a major strength of our trial. The results of single-centre trials have in several cases not been reproduced in multicentre settings.[47] Due to the substantial variations in clinical practice regarding use of resuscitation fluids, a multi-centre setting was indeed preferable to indicate general feasibility. Even in the multicentre setting of CLASSIC, there is a risk of selection bias of sites who had clinical staff and investigators who found a protocol of fluid restriction intriguing.

Patient selection

We chose to assess fluid resuscitation volumes in patients with septic shock rather than severe sepsis (as defined by ACCP/SCCM[48]), because the requirement of vasopressors to maintain adequate arterial blood pressure indicates hemodynamic comprise which increases the probability of fluid administration.[48] Thus, we expected the intervention effect on fluid volumes to be larger in patients with septic shock, since administration of vasopressor and increasing dose of vasopressors were not valid criteria for fluid resuscitation in the restrictive group. The caveat was that the results of the trial may not be generalizable to patients with sepsis without shock. We added an inclusion criterion stating that patients had to have received at least 30 ml/kg of fluid within 6 hours of randomisation for mainly two reasons. First, to prevent randomisation of dehydrated patients. Second, to adhere to the 2012 SSC recommendation of an initial

fluid challenge.[20] Since 30 ml/kg within 6 hours was a minimum, the median volumes administered 24 hours prior to randomisation were substantially higher. This inferred that some patients may have been fully resuscitated at randomisation, which would tend to lessen the protocol effect on fluid volumes, but the criterion might also have reduced protocol violations. We included only patients who had severe circulatory impairment for less than 12 hours, including the hours preceding ICU admission. Initially, we defined circulatory impairment based on several clinical parameters, but we found this definition to be unsuitable after commencing the trial, because of challenges with finding sufficient documentation in the patient charts (e.g. for capillary refill time) and examples of patients, who were otherwise relevant to enrol, fulfilled one criterion for circulatory impairment for more than 12 hours. The revised definition was based on criteria for acquiring a medical response team (MRT);[49] this protocol revision was applied for after only 7 patients were enrolled and is unlikely to have affected the results noteworthy.

We excluded patients with severely impaired kidney function, who had received or were at high risk of receiving RRT soon after randomisation; this was primarily chosen, because RRT inevitably complicates a fluid input protocol. After randomisation, the protocol was continued in case of RRT initiation, but we aimed at minimising the proportion of patients with little or no time without RRT following randomisation. We also excluded patients with severe hypoxic failure; this was chosen because we anticipated a more restrictive approach to fluid administration in these patients as standard care. Thus, the protocol would likely to have had smaller effect in these patients. The choices of excluding patients with severe hypoxic and kidney failure infer that the results may not be generalizable to these groups of patients.

The consent procedures allowed fast randomisation of patients of all illness severities; in Denmark, informed consent could be obtained from two independent doctors and in Finland, the use of deferred consent was approved by the ethics committee.

Interventions

The intervention groups were thoroughly discussed in the planning of the trial. We assembled a group of investigators from all centres who had expressed interest in participating in the trial with the aim of increasing protocol acceptance across multiple trial sites. We chose four criteria for fluid resuscitation in the restrictive group – we considered these markers of severe hypoperfusion. Especially the lactate criterion merits discussion. The lactate thresholds of 4 mM was chosen because lactate values above ~4mM has been associated with increased mortality in septic shock whereas mortality was similar for values between ~2 and ~4 mM.[50] Interpretation of plasma lactate is complex in septic shock and several factors may contribute to increased values even in case of adequate perfusion, including adrenergic-induced glycolysis, mitochondrial dysfunction, impaired activity of pyruvate dehydrogenase, and liver failure.[51] Presently, however, we cannot derive the exact aetiology of hyperlactatemia in clinical practice, and the choice of 4 mM threshold was based on the hypothesis that a substantial proportion of lactate values below 4 were not due to inadequate perfusion, and therefore, the potential benefits from fluid resuscitation would not outweigh the adverse effects. Importantly, fulfilment of at least one criterion in the fluid restriction group did not mandate fluid resuscitation, but was a prerequisite. The results must,

therefore, be interpreted accordingly, and even in the case of the results being reproduced in a future large-scale trial, no strict algorithm for fluid resuscitation can be deduced – clinical judgement would still need to accompany the objective criteria.

The 'control group' also underwent discussion and was narrowed down to three possibilities: a standard care protocol, usual care (i.e. no protocol), or a goal-directed protocol. The goal-directed protocol – e.g. assisted by dynamic measures of fluid responsiveness[52] – was dismissed, because of the apparent infrequent use in daily clinical practice;[48,53] and comparing two protocols where neither reflected daily practice would limit the clinical applicability of the results. Of note, a single-centre trial comparing a preload indices-guided fluid strategy with a CVP-guided fluid strategy has been performed in 60 patients with septic shock in France; the daily amount of fluids administered for volume expansion were lower in the preload dependence group, but the primary outcome of time to resolution of shock did not differ between the two groups.[54] The usual care arm was also dismissed due to the reduced comparability with regards to fluid resuscitation volumes; fluids administered as maintenance or with nutrition and medications are likely to have a different balance between benefits and harms compared to fluid resuscitation, and the intended comparison of fluid resuscitation volumes would be impeded by the lack of differentiation of fluid input in a usual care arm. We chose a standard care protocol where the recommendations for fluid administration other than fluid resuscitation were equal to that of the fluid restrictive group – thus the only difference between the two intervention groups was the indications for fluid resuscitation. This choice might have inferred decreased protocol effect on fluid volumes with decreased fluid resuscitation volumes in the standard care group compared to a usual care group without a protocol. On the other hand, the strict comparison of fluid resuscitation volumes strengthened the conclusions. Additionally, we may have been exposed to an observer effect (a Hawthorne effect), i.e. we might have changed practice merely by questioning and monitoring fluid volumes.[55]

In both intervention groups, administration of colloid solutions for circulatory impairment was regarded as a protocol violation. In a recent observational study, albumin was the only colloid used in Denmark for fluid bolus therapy (unpublished data from Fluid-TRIPS; clinicaltrials.gov identifier: NCT02002013, manuscript submitted). Thus, we acknowledged the potential for protocol violations with use of albumin. Despite the risk of protocol violations, we chose to protocolise against the use of albumin due to the risk of between-group differences in the use of albumin after randomisation, i.e. an expectation of increased use of albumin in the fluid restriction group due to perceived greater intravascular volume expansion effect. We did not consider this to be of risk to participating patients as two large-scale randomised trials did not find a conclusive beneficial effect of albumin.[56,57]

Outcomes

The primary and secondary outcome measures reflected the feasibility trial design with special emphasis on fluid volumes. During the trial, the secondary outcome measure – volume of resuscitation fluid given during ICU stay – was promoted to a co-primary outcome measure. Changing the primary outcome of a trial has

been criticised and has been associated with industry funding.[58] Our decision to promote the outcome was, therefore, not straightforward and was carefully discussed. We decided to promote the outcome measure, because the intervention period was entire ICU stay, and we wanted this to be reflected in a primary outcome. The predefined primary outcome measure in the protocol – volume of resuscitation fluid first 5 days after randomisation – was kept as a co-primary outcome measure. We considered this to be the most valid measure for between-group comparison, because it was less likely to be affected by length of ICU stay as compared to the measure for entire ICU stay.

Total fluid inputs did not differ with statistical significance between the two groups. These results from the CLASSIC trial have been suggested to imply that clinicians will resort to other fluid therapies when fluid bolus therapy is not an option.[59] The point estimates for the between-group differences, however, were similar to those of fluid resuscitation volumes suggesting that the lack of statistical significance was due to the large variations observed – and expected – with total fluid inputs. The pre-trial power estimations for the secondary outcome measures presented in the protocol showed that the detectable differences for total fluid inputs were higher than those of fluid resuscitation volumes (3.9 vs. 1.7 L and 19.7 vs. 3.7 L for entire ICU stay and first five days after randomisation, respectively). Since we only intervened on fluid resuscitation, significant differences between the two groups were not expected for total fluid inputs. Nevertheless, due to the large variations in total fluid inputs, it cannot be refuted that patients in the fluid restriction group received higher volumes of non-resuscitation fluids compared with the standard care group during the trial. Similarly, the cumulated fluid balance the first 5 days after randomisation did not differ between the two groups with statistical significance, but the point estimate was similar to that of fluid resuscitation volume.

The patient-centred exploratory outcomes all favoured fluid restriction, but we were not powered to show any differences in these outcomes. The proportion of patients with worsening of AKI according to the KDIGO criteria[60] appeared to be lower in the fluid restriction group, but conclusions should be drawn with cautions due to the risk of a type 1 error and possible baseline imbalances as a consequence of the limited sample size. Also, the other pre-defined outcome measures concerning kidney impairment did not appear to be favoured with similar magnitude, although the point estimates all were in favour of fluid restriction.

In the fluid restriction group, the protocol was violated with administration of fluid resuscitation without fulfilment of at least one of the criteria during entire ICU stay in 80 fluid episodes in about one third of the patients. Just 20 of these protocol violations in one sixth of the patients occurred in the first 24 hours after randomisation (presented in Study III). If hemodynamic variables were the only determinants for violating the protocol, we would expect that the majority of the protocol violations would have occurred early where the hemodynamic compromise was more pronounced. The observation that the bulk of the protocol violations occurred beyond the first 24 hours suggests that other factors were likely to have contributed. Arguably, an important aspect is the time at risk – i.e. the longer time the patient was admitted to the ICU, the longer time the patient was at risk of having the protocol violated. The protocol violations decreased the internal validity of the results, and reducing the number of protocol violations in a future large-scale trial is of paramount importance. During the CLASSIC trial, we did not argue against protocol violations

since we did not have any data on the safety of our protocol, but we encouraged the clinicians to provide reasons if they judged a protocol violation pertinent. In a large-scale trial, we can use the results of the CLASSIC trial to argue against protocol violations which might reduce the rate, but a violation of the protocol will always be at the discretion of treating clinicians. As expected, the majority of protocol violations were related to the CLASSIC criteria (vasopressors/MAP and urinary output), but we had not expected that high heart rate would be one of the leading reasons for violating the protocol. If high heart rate was introduced as valid criterion for fluid resuscitation, the number of protocol violations might be reduced, but the intervention effect on fluid volumes might be decreased. The effect of fluid administration for tachycardia in septic shock on organ perfusion is complex and not fully elucidated; there is a potential beneficial effect if the fluid reduces the heart rate and the reduced heart rate leads to increased ventricular filling time and improves the supply/demand of oxygen to the myocardium. On the other hand, there is a risk that a considerable proportion of these patients will not respond to fluid administration with improved organ perfusion.

Statistics

The statistical analyses in Study II were performed according to a pre-defined statistical analysis plan which was a major strength. In our primary analysis we tested for group differences adjusted for trial site (the stratification variable) in the co-primary outcome measures using a generalised linear model. We adjusted for the stratification variable, because the stratified randomisation leads to correlated treatment groups and ignoring this correlation may lead to too wide 95% confidence intervals, too low type 1 error rates and reduced statistical power.[61,62] Sensitivity analyses where we adjusted the analysis of the co-primary outcomes for pre-defined baseline characteristics presumed to be associated with increased fluid resuscitation volumes supported the results of the primary analyses. Adjusted analyses are prone to bias if the adjusting variables are chosen post hoc and data-driven,[63] and the item 12b of the CONSORT guidelines recommends against this practice.[64]

Intention-to-treat analysis where all randomised patients are analysed according to their original group assignment is the gold standard for the analysis of randomised clinical trials. We performed all analysis as intention-to-treat, but according to national law, we excluded patients who withdrew consent to the use of data (one patient in each group in our trial). Post randomisation exclusions may lead to reduced statistical power if the patients are not replaced by additional randomisations, and systematic errors as the dropouts may be related to the outcome and eventually affect the results and conclusion of the trial.[65] Additionally, the use of delayed informed consent and informed consent by proxy increased the risk of post-randomisation withdrawal from the intervention as sometimes patients and/or relatives requested to stop the ongoing trial (two patients in each group in our trial), which may lead to shortened intervention period with reduction of group differences.

The use of co-primary outcome measures inferred multiplicity issues. To account for multiple testing for differences between the intervention groups in the co-primary outcome measures, we adjusted the

significance level by dividing with a factor of 1.5. We chose a factor between no adjustment (a factor of 1) and a full Bonferroni adjustment (a factor of 2), because we anticipated a degree of correlation between the outcome measures. Arguably, the correlation was substantial since the outcome measures only differed by observation period, and a factor of 1.5 was therefore a conservative choice.

Limitations and strengths – the post hoc analysis of the CLASSIC trial (Study III)

The post hoc analysis of the CLASSIC trial shared most of the strengths and limitations of the CLASSIC trial, but one important distinction merits discussion. The post hoc analysis was not predefined, and the decision to perform the analyses was made after the results of the CLASSIC trial were known. To illustrate the pitfalls with post hoc analyses, we have previously published an article that may serve as an exaggerated example of caveats of post hoc analyses and data-driven hypothesis generation. We compared the mortality for patients born under the zodiac sign Pisces with patients born under other zodiac signs in the 6S trial database.[66] We found that Pisces had lower 90-day mortality compared to other zodiac signs, and the difference was statistically significant with a one-sided p-value of 0.03. Interestingly, we observed that Pisces had higher SAPS II than the other zodiac, and when we adjusted for this confounder, the difference was statistically significant with a two-sided p-value of 0.01. Many methodological flaws were deliberately made in that study and the hypothesis was intendedly implausible, but it serves as an example of the extent of flawed conclusions that are more likely when performing analyses which are not predefined.

We defined the analyses in Study III prior to accessing the CLASSIC trial database, but the results of the CLASSIC trial where known; thus, the results should be confirmed in other studies before firm conclusions can be drawn.

Current evidence for the use of fluid resuscitation in septic shock

The 2016 Surviving Sepsis Campaign guidelines provided recommendations for fluid resuscitation in septic shock in mainly two parts regarding fluid volumes: the initial challenge of at least 30 ml/kg, and a recommendation for continued fluid therapy where fluid resuscitation was recommended as long as the circulation improves,[31] which – apart from the omission of the 6-hour EGDT bundle – were largely unchanged from the 2012 guidelines.[20] The studies in the present thesis challenged mainly the recommendation for continued fluid therapy. A high-quality systematic review with meta-analyses by Silversides and colleagues systematically searched the literature for studies on conservative vs. liberal fluid management after the initial resuscitation in ICU patients with sepsis and acute respiratory distress syndrome (ARDS).[67] Despite the broad inclusion criteria, they found a total of 11 RCTs (3 in adult patients with septic shock). The meta-analysis of the included RCTs found a relative risk of mortality for a conservative vs. a liberal strategy of 0.92 (95% CI 0.82-1.02), indicating potential – but not evident – harm with higher fluid volumes. Patients assigned to a conservative fluid strategy had more ventilator free days

(VFD) compared with patients assigned to a liberal strategy; this results was largely driven by the FACTT trial.[37] The FEAST trial found increased 48-hour mortality with a fluid bolus of 0.9% saline or albumin compared with no fluid bolus in febrile African children with circulatory impairment.[36] The generalisability of these findings does not extend to adults with septic shock in high-resource countries; nevertheless, they do raise concern with current practice and guidelines where fluid resuscitation is promoted. Interestingly, in a post hoc analysis, the authors reported a larger proportion of children with alleviated circulatory impairment one hour after randomisation in the bolus groups despite them having higher 48-hour mortality,[68] thereby suggesting initial circulatory improvement, but worse short-term outcome. Also, most observational studies have indicated harm with increasing fluid balances and input,[33-35] but analyses without statistically significant differences have been reported as well.[69] The efficacy of fluid resuscitation beyond an initial effect is less studied[70], but a recent observational study where the majority of patients had septic shock found that the initial increase in cardiac index observed in fluid responders was not sustained and the cardiac index had returned to baseline after 90 minutes.[71] The results of Study III were in accordance with this finding, further questioning the efficacy of fluid resuscitation in septic shock beyond the initial fluid management. Additionally, a theoretic physiological mechanism for the reduced efficacy of fluid resuscitation after the initial management have been suggested with increased mean circulatory filling pressures leading to increased force for capillary filtration.[72]

Taken together, there is evidence to suggest that higher volumes of resuscitation fluid are harmful even within the range of volumes administered in clinical practice. To our knowledge, we were the first to successfully randomise patients with septic shock in multiple centres to higher vs. lower volumes of resuscitation fluid, and the results of the exploratory outcome measures added to the growing body of literature indicating harm with higher volumes.

There is currently no conclusive evidence available to make firm recommendations to guide treating clinicians in daily practice, and the decisions concerning fluid resuscitation must, therefore, still be based on the challenging task of combining physiological rationale with an interpretation of the available clinical data. Accordingly, controversy remains with some arguing that early liberal fluid therapy in sepsis is harmful[73] while others argue that it is not harmful,[74] and many questions remain to be answered before firm recommendations for fluid management can be provided.[75]

Fluid resuscitation and acute kidney injury

We reported fewer patients with worsening of AKI in the fluid restriction group in the CASSIC trial and in Study III, we suggested that fluid resuscitation might not increase the urine output. Although urine output is a frequent indication for fluid administration,[48,53] and the majority of respondents in a global survey of intensivist considered a positive response to a fluid bolus as an increase of at least 20 ml/hour,[76] there is limited data to support this practice, and high-quality data from RCTs on fluid volumes in AKI are sparse. The physiological rationale for fluid administration in case of 'absolute hypovolemia' – i.e. dehydration – has not been questioned, but it may have led to the classical notion that low urine output is due to hemodynamic compromise leading to decreased renal perfusion and that fluid administration will alleviate

the condition. This is likely to be oversimplified – especially in case of the septic AKI which has been proposed to be associated with maintained or increased renal blood flow rather than impaired renal perfusion.[77-80] The systematic review and meta-analysis by Silversides and colleagues found no statistically significant difference in use of RRT for conservative vs. liberal strategies (risk ratio 0.88; 95% CI 0.64-1.22), but the analysis was characterized by imprecision as only three trials were included.[67] In the 3-armed PROCESS trial, patients with septic shock randomised to the protocol-based standard therapy group received more fluids and had higher risk of new onset renal failure compared with patients randomised to the EGDT group and the usual care group.[23] Of note, fluids were only one part of the intervention in the trial which also included vasopressors, dobutamine and blood transfusions. Observational studies have indicated harm with increased fluid balance in AKI[81-83] and in patients receiving renal replacement therapy.[84] Even though these analyses were adjusted for illness severity, conclusions must be drawn with caution due to risk of confounding, and inference about causality cannot be made since the increased fluid balance might be indicative of illness severity not reflected in the summary scores that were used to perform the adjustments.

Thus, apart from our findings, there is evidence to suggest that higher fluid inputs may precipitate rather than alleviate AKI, but no firm conclusions can be made from the available data. AKI comprise a broad spectrum of pathophysiological characteristics, and a 'one size fits all'-approach regarding fluid input is unlikely to be obtainable, but a hesitant approach to persistent fluid administration aiming at increasing the urinary output is likely to be prudent. Intravenous fluids are drugs, which has also been emphasised in the recent literature.[85] Taking this argument further, the pharmacokinetic clearance of IV fluids is primarily through renal excretion; thus, administration of fluids in case of impaired excretion must be performed with careful consideration to whether the potential beneficial effects outweigh the increased risk of adverse effects.

CONCLUSIONS AND FUTURE PERSPECTIVES

The CLASSIC trial was the first trial to randomise adult patients with septic shock to a protocol with the specific aim of reducing volumes of resuscitation fluid and a protocol aiming at standard care and achieve between-group separation in fluid volumes. The CLASSIC trial illustrated feasibility, but also safety – at least in an RCT setting – because all point-estimates for the patient-centred outcome measures favoured fluid restriction. The results of the post hoc analysis of the CLASSIC trial suggested lack of sustained efficacy with fluid resuscitation. The necessity of conducting further research was emphasised in the observational analysis of the 6S trial database where the results indicated variations in the use of fluid resuscitation between ICUs.

Thus, the studies in the present thesis have illustrated both the feasibility and emphasised the importance of conducting a large-scale trial. A protocol similar to that of CLASSIC would be ideal in order to maximise the applicability of the CLASSIC trial findings, but fluid therapy in septic shock is complex and one large-scale trial is not sufficient to establish high-quality evidence-based fluid therapy in septic shock. We need several trials with different protocols to achieve this goal, and we need further physiological studies and trials to improve the understanding of the hemodynamic consequences of fluid resuscitation and their durations. This will not be an easy task, but it is important to future patients that we improve our knowledge on one of the most frequent interventions in medicine that has been administered to millions of patients across the world for decades.

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REFERENCES

1. Wiles JB, Cerra FB, Siegel JH, Border JR. The systemic septic response: does the organism matter? *Crit. Care Med.* 1980;8(2):55-60.
2. Bone RC, Balk RA, Cerra FB, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest.* 1992;101(6):1644-1655.
3. Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA.* 2016;315(8):801-810.
4. Vincent JL, Moreno R, Takala J, et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. *Intensive Care Med.* 1996;22(7):707-710.
5. Angus DC, van der Poll T. Severe sepsis and septic shock. *N. Engl. .J Med.* 2013;369(9):840-851.
6. Gotts JE, Matthay MA. Sepsis: pathophysiology and clinical management. *BMJ.* 2016;353:i1585.
7. Melo J, Peters JI. Low systemic vascular resistance: differential diagnosis and outcome. *Crit. Care.* 1999;3(3):71-77.
8. Parker MM, Shelhamer JH, Bacharach SL, et al. Profound but reversible myocardial depression in patients with septic shock. *Ann. Intern. Med.* 1984;100(4):483-490.
9. Weisel RD, Vito L, Dennis RC, Valeri CR, Hechtman HB. Myocardial depression during sepsis. *Am. J. Surg.* 1977;133(4):512-521.
10. Karlsson S, Ruokonen E, Varpula T, Ala-Kokko TI, Pettilä V. Long-term outcome and quality-adjusted life years after severe sepsis. *Crit. Care Med.* 2009;37(4):1268-1274.
11. Pettila V, Hjortrup PB, Jakob SM, Wilkman E, Perner A, Takala J. Control groups in recent septic shock trials: a systematic review. *Intensive Care Med.* 2016;42(12):1912-1921.
12. Lagu T, Rothberg MB, Shieh MS, Pekow PS, Steingrub JS, Lindenauer PK. Hospitalizations, costs, and outcomes of severe sepsis in the United States 2003 to 2007. *Crit. Care Med.* 2012;40(3):754-761.
13. Mackintosh J. *Principles of Pathology and Practice of Physic.* Vol 1. 3 ed. Philadelphia: Key & Briddle; 1836.
14. O'Shaugnessy WB. Proposal of a new method of treating the blue epidemic cholera by the injection of highly oxygenised salts in to the venous system. *Lancet.* 1831;1:366-371.
15. Latta T. Malignant Cholera. *Lancet.* 1832;18(460):274-280.
16. Patterson SW, Starling EH. On the mechanical factors which determine the output of the ventricles. *J. Physiol.* 1914;48(5):357-379.
17. Weil MH, Shubin H, Rosoff L. Fluid Repletion in Circulatory Shock: Central Venous Pressure and Other Practical Guides. *JAMA.* 1965;192:668-674.
18. Weil MH, Shubin H. Treatment of shock caused by bacterial infections. *Calif. Med.* 1973;119(5):7-13.
19. Rivers E, Nguyen B, Havstad S, et al. Early goal-directed therapy in the treatment of severe sepsis and septic shock. *N. Engl. J. Med.* 2001;345(19):1368-1377.
20. Dellinger RP, Levy MM, Rhodes A, et al. Surviving sepsis campaign: International guidelines for management of severe sepsis and septic shock, 2012. *Intensive Care Med.* 2013;39(2):165-228.
21. Jones AE, Shapiro NI, Trzeciak S, Arnold RC, Claremont Ha, Kline Ja. Lactate clearance vs central venous oxygen saturation as goals of early sepsis therapy: a randomized clinical trial. *JAMA.* 2010;303(8):739-746.
22. Jansen TC, van Bommel J, Schoonderbeek FJ, et al. Early lactate-guided therapy in intensive care unit patients: a multicenter, open-label, randomized controlled trial. *Am. J. Respir. Crit. Care Med.* 2010;182(6):752-761.
23. Yealy DM, Kellum JA, Huang DT, et al. A Randomized Trial of Protocol-Based Care for Early Septic Shock. *N. Engl. .J Med.* 2014; 370(18): 1683-1693.
24. Peake SL, Delaney A, Bailey M, et al. Goal-directed resuscitation for patients with early septic shock. *N. Engl. J. Med.* 2014;371(16):1496-1506.

25. Mouncey PR, Osborn TM, Power GS, et al. Trial of early, goal-directed resuscitation for septic shock. *N. Engl. J. Med.* 2015;372(14):1301-1311.
26. Angus DC, Barnato aE, Bell D, et al. A systematic review and meta-analysis of early goal-directed therapy for septic shock: the ARISE, ProCESS and ProMISe Investigators. *Intensive Care Med.* 2015;41(9):1549-1560.
27. Marik PE, Cavallazzi R. Does the central venous pressure predict fluid responsiveness? An updated meta-analysis and a plea for some common sense. *Crit. Care Med.* 2013;41(7):1774-1781.
28. Eskesen TG, Wetterslev M, Perner A. Systematic review including re-analyses of 1148 individual data sets of central venous pressure as a predictor of fluid responsiveness. *Intensive Care Med.* 2016;42(3):324-332.
29. Rhodes A, Phillips G, Beale R, et al. The Surviving Sepsis Campaign bundles and outcome: results from the International Multicentre Prevalence Study on Sepsis (the IMPreSS study). *Intensive Care Med.* 2015;41(9):1620-1628.
30. Levy MM, Rhodes A, Phillips GS, et al. Surviving Sepsis Campaign: association between performance metrics and outcomes in a 7.5-year study. *Intensive Care Med.* 2014;40(11):1623-1633.
31. Rhodes A, Evans LE, Alhazzani W, et al. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. *Intensive Care Med.* 2017.
32. Chappell D, Bruegger D, Potzel J, et al. Hypervolemia increases release of atrial natriuretic peptide and shedding of the endothelial glycocalyx. *Crit. Care.* 2014;18(5):538.
33. Boyd JH, Forbes J, Nakada T-a, Walley KR, Russell JA. Fluid resuscitation in septic shock: a positive fluid balance and elevated central venous pressure are associated with increased mortality. *Crit. Care Med.* 2011;39(2):259-265.
34. Vincent J-L, Sakr Y, Sprung CL, et al. Sepsis in European intensive care units: results of the SOAP study. *Crit. Care Med.* 2006;34(2):344-353.
35. Acheampong A, Vincent J-L. A positive fluid balance is an independent prognostic factor in patients with sepsis. *Crit. Care.* 2015;19(1):251-251.
36. Maitland K, Kiguli S, Opoka RO, et al. Mortality after Fluid Bolus in African Children with Severe Infection. *N. Engl. J. Med.* 2011;364(26):2483-2495.
37. Wiedemann HP, Wheeler AP, Bernard GR, et al. Comparison of two fluid-management strategies in acute lung injury. *N. Engl. J. Med.* 2006;354(24):2564-2575.
38. 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(2):124-134.
39. 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. *Trials.* 2011;12(1):24-24.
40. Le Gall JR, Lemeshow S, Saulnier F. A new Simplified Acute Physiology Score (SAPS II) based on a European/North American multicenter study. *JAMA.* 1993;270(24):2957-2963.
41. Crowe BJ, Lipkovich IA, Wang O. Comparison of several imputation methods for missing baseline data in propensity scores analysis of binary outcome. *Pharm. Stat.* 2010;9(4):269-279.
42. Ait-Oufella H, Lemoinne S, Boelle PY, et al. Mottling score predicts survival in septic shock. *Intensive Care Med.* 2011;37(5):801-807.
43. Livingston E, Cao J, Dimick JB. Tread carefully with stepwise regression. *Arch. Surg.* 2010;145(11):1039-1040.
44. Everitt B. *Medical Statistics from A to Z: A Guide for Clinicians and Medical.* 2 ed: Students Cambridge University Press; 2006.
45. Lancaster GA, Dodd S, Williamson PR. Design and analysis of pilot studies: recommendations for good practice. *J. Eval. Clin. Pract.* 2004;10(2):307-312.
46. Chen C, Kollef MH. Targeted Fluid Minimization Following Initial Resuscitation in Septic Shock: A Pilot Study. *Chest.* 2015;148(6):1462-1469.
47. Bellomo R, Warrillow SJ, Reade MC. Why we should be wary of single-center trials. *Crit. Care Med.* 2009;37(12):3114-3119.
48. Bihari S, Baldwin CE, Bersten AD, et al. Post resusicitation fluid boluses in severe sepsis or septic shock: prevalence and efficacy (price study). *Crit. Care Resusc.* 2013;12(1):66-70.

49. Hillman K, Chen J, Cretikos M, et al. Introduction of the medical emergency team (MET) system: a cluster-randomised controlled trial. *Lancet.* 2005;365(9477):2091-2097.
50. Wacharasint P, Nakada T-a, Boyd JH, Russell Ja, Walley KR. Normal-range blood lactate concentration in septic shock is prognostic and predictive. *Shock (Augusta, Ga.).* 2012;38(1):4-10.
51. Jansen TC, van Bommel J, Bakker J. Blood lactate monitoring in critically ill patients: a systematic health technology assessment. *Crit. Care Med.* 2009;37(10):2827-2839.
52. Vincent J-L, Weil MH. Fluid challenge revisited. *Crit. Care Med.* 2006;34(5):1333-1337.
53. Cecconi M, Hofer C, Teboul J-L, et al. Fluid challenges in intensive care: the FENICE study : A global inception cohort study. *Intensive Care Med.* 2015;41(9):1529-1537.
54. Richard JC, Bayle F, Bourdin G, et al. Preload dependence indices to titrate volume expansion during septic shock: a randomized controlled trial. *Crit. Care.* 2015;19:5.
55. McCambridge J, Witton J, Elbourne DR. Systematic review of the Hawthorne effect: new concepts are needed to study research participation effects. *J. Clin. Epidemiol.* 2014;67(3):267-277.
56. Finfer S, Bellomo R, Boyce N, French J, Myburgh J, Norton R. A comparison of albumin and saline for fluid resuscitation in the intensive care unit. *The New England journal of medicine.* 2004;350(22):2247-2256.
57. Caironi P, Tognoni G, Masson S, et al. Albumin Replacement in Patients with Severe Sepsis or Septic Shock. *N. Engl. J. Med.* 2014; 370(15): 1412-1421.
58. Ramagopalan S, Skingsley AP, Handunnetthi L, et al. Prevalence of primary outcome changes in clinical trials registered on ClinicalTrials.gov: a cross-sectional study. *F1000Res.* 2014;3:77.
59. Glassford NJ, Bellomo R. The Complexities of Intravenous Fluid Research: Questions of Scale, Volume, and Accumulation. *Korean J Crit Care Med.* 2016;31(4):276-299.
60. Group KAKIW. KDIGO Clinical Practice Guideline for Acute Kidney Injury. *Kidney International Supplements.* 2012;2(1):1-138.
61. Kahan BC, Morris TP. Improper analysis of trials randomised using stratified blocks or minimisation. *Stat. Med.* 2012;31(4):328-340.
62. Kahan BC, Morris TP. Reporting and analysis of trials using stratified randomisation in leading medical journals: review and reanalysis. *BMJ.* 2012;345:e5840.
63. Kahan BC, Jairath V, Dore CJ, Morris TP. The risks and rewards of covariate adjustment in randomized trials: an assessment of 12 outcomes from 8 studies. *Trials.* 2014;15:139.
64. Moher D, Hopewell S, Schulz KF, et al. CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. *BMJ (Clinical research ed.).* 2010;340:c869-c869.
65. Deeks JJ, Dinnes J, D'Amico R, et al. Evaluating non-randomised intervention studies. *Health Technol. Assess.* 2003;7(27):iii-x, 1-173.
66. Hjortrup PB, Haase N, Wetterslev J, Perner A. Gone fishing in a fluid trial. *Crit. Care Resusc.* 2016;18(1):55-58.
67. Jonathan A Silversides EM, Andrew J Ferguson, Emma E Mann, Daniel F McAuley, John, C Marshall BB, Eddy Fan. Conservative fluid management or deresuscitation for patients with sepsis or acute respiratory distress syndrome following the resuscitation phase of critical illness: a systematic review and meta-analysi. *Intensive Care Med.* 2016.
68. Maitland K, George EC, Evans Ja, et al. Exploring mechanisms of excess mortality with early fluid resuscitation: insights from the FEAST trial. *BMC Med.* 2013;11:68-68.
69. Cronhjort M, Hjortrup PB, Holst LB, et al. Association between fluid balance and mortality in patients with septic shock: a post hoc analysis of the TRISS trial. *Acta Anaesthesiol. Scand.* 2016;60(7):925-933.
70. Glassford NJ, Eastwood GM, Bellomo R. Physiological changes after fluid bolus therapy in sepsis: a systematic review of contemporary data. *Crit. Care.* 2014;18(6):696.
71. Nunes TS, Ladeira RT, Bafi AT, de Azevedo LC, Machado FR, Freitas FG. Duration of hemodynamic effects of crystalloids in patients with circulatory shock after initial resuscitation. *Ann Intensive Care.* 2014;4:25.
72. Magder S. Volume and its relationship to cardiac output and venous return. *Crit. Care.* 2016;20:271.
73. Genga K, Russell JA. Early Liberal Fluids for Sepsis Patients Are Harmful. *Crit. Care Med.* 2016;44(12):2258-2262.

74. Jaehne AK, Rivers EP. Early Liberal Fluid Therapy for Sepsis Patients Is Not Harmful: Hydrophobia Is Unwarranted but Drink Responsibly. *Crit. Care Med.* 2016;44(12):2263-2269.
75. Hjortrup PB, Delaney A. Fluid management in the ICU: has the tide turned? *Intensive Care Med.* 2016.
76. Glassford NJ, Mårtensson J, Eastwood GM, et al. Defining the characteristics and expectations of fluid bolus therapy: A worldwide perspective. *J. Crit. Care.* 2016;35.
77. Wan L, Bagshaw SM, Langenberg C, Saotome T, May C, Bellomo R. Pathophysiology of septic acute kidney injury: what do we really know? *Crit. Care Med.* 2008;36(4 Suppl):S198-203.
78. Langenberg C, Bellomo R, May C, Wan L, Egi M, Morgera S. Renal blood flow in sepsis. *Crit. Care.* 2005;9(4):R363-374.
79. Langenberg C, Bellomo R, May CN, Egi M, Wan L, Morgera S. Renal vascular resistance in sepsis. *Nephron Physiol.* 2006;104(1): 1-11.
80. Brenner M, Schaer GL, Mallory DL, Suffredini AF, Parrillo JE. Detection of renal blood flow abnormalities in septic and critically ill patients using a newly designed indwelling thermodilution renal vein catheter. *Chest.* 1990;98(1):170-179.
81. Teixeira C, Garzotto F, Piccinni P, et al. Fluid balance and urine volume are independent predictors of mortality in acute kidney injury. *Crit. Care.* 2013;17(1):R14.
82. Payen D, de Pont AC, Sakr Y, Spies C, Reinhart K, Vincent JL. A positive fluid balance is associated with a worse outcome in patients with acute renal failure. *Crit. Care.* 2008;12(3):R74.
83. Bouchard J, Soroko SB, Chertow GM, et al. Fluid accumulation, survival and recovery of kidney function in critically ill patients with acute kidney injury. *Kidney Int.* 2009;76(4):422-427.
84. Vaara ST, Korhonen A-M, Kaukonen K-M, et al. Fluid overload is associated with an increased risk for 90-day mortality in critically ill patients with renal replacement therapy: data from the prospective FINNAKI study. *Crit. Care.* 2012;16(5):R197.
85. Raghunathan K, Shaw AD, Bagshaw SM. Fluids are drugs: type, dose and toxicity. *Curr. Opin. Crit. Care.* 2013;19(4):290-298.

PAPER I

PLOS ONE

RESEARCH ARTICLE

Associations of Hospital and Patient Characteristics with Fluid Resuscitation Volumes in Patients with Severe Sepsis: Post Hoc Analyses of Data from a Multicentre Randomised Clinical Trial

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Abstract

Purpose

Fluid resuscitation is a key intervention in patients with sepsis and circulatory impairment. The recommendations for continued fluid therapy in sepsis are vague, which may result in differences in clinical practice. We aimed to evaluate associations between hospital and patient characteristics and fluid resuscitation volumes in ICU patients with severe sepsis.

Methods

We explored the 6S trial database of ICU patients with severe sepsis needing fluid resuscitation randomised to hydroxyethyl starch 130/0.42 vs. Ringer's acetate. Our primary outcome measure was fluid resuscitation volume and secondary outcome total fluid input administered from 24 hours before randomisation until the end of day 3 post-randomisation. We performed multivariate analyses with hospital and patient baseline characteristics as covariates to assess associations with fluid volumes given.

Results

We included 654 patients who were in the ICU for 3 days and had fluid volumes available. Individual trial sites administered significantly different volumes of fluid resuscitation and total fluid input after adjusting for baseline variables (P<0.001). Increased lactate, higher cardiovascular and renal SOFA subscores, lower respiratory SOFA subscore and surgery were all independently associated with increased fluid resuscitation volumes.

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Conclusions

Hospital characteristics adjusted for patient baseline values were associated with differences in fluid resuscitation volumes given in the first 3 days of severe sepsis. The data indicate variations in clinical practice not explained by patient characteristics emphasizing the need for RCTs assessing fluid resuscitation volumes fluid in patients with sepsis.

Introduction

For decades fluid resuscitation has been considered a pivotal intervention in the treatment of patients with sepsis and circulatory impairment. The hemodynamic consequences of sepsis are complex and several pathophysiological characteristics serve as rationale for fluid administration including dehydration, increased vascular permeability leading to decreased intravascular fluid volume [1] and decreased vascular tone [2]. However, fluid administration also has potential adverse effects including increasing tissue edema [3] and electrolyte derangements [4, 5].

The optimal volume of fluid and indications for fluid resuscitation in severe sepsis are not established. Fluid resuscitation guided by central venous pressure in the first six hours of septic shock was a part of the protocol in the landmark trial of Early Goal-Directed Therapy (EGDT) in sepsis by Rivers et al that showed significantly increased survival with EGDT [6]. Since then, three large-scale multicentre trials have reported no benefit of EGDT vs. standard care [7–9], but the use of resources was increased with EGDT [10]. Beyond the first six hours the international guidelines for fluid resuscitation are vague and ungraded due to lack of evidence; the 2012 recommendation for continued fluid therapy from the Surviving Sepsis Campaign states: "Fluid challenge technique be applied wherein fluid administration is continued as long as there is hemodynamic improvement either based on dynamic (e.g. change in pulse pressure, stroke volume variation) or static (e.g. arterial pressure, heart rate) variables" [11].

The lack of firm evidence and vague recommendations may infer differences in clinical practice. The aim of this study was to investigate whether hospital characteristics adjusted for patient baseline characteristics were associated with differences in fluid resuscitation volumes in a multicentre randomised clinical trial in patients with severe sepsis needing fluid resuscitation. Furthermore, we aimed to describe patient baseline characteristics' association with fluid resuscitation volumes.

Methods

This was a retrospective analysis of data from the Scandinavian Starch for Severe Sepsis and Septic Shock (6S) trial. In the 6S trial adult patients with severe sepsis and need of fluid resuscitation were randomised to resuscitation with hydroxyethyl starch (HES) 130/0.42 (Tetraspan, B Braun Medical AG, Melsungen, Germany) or Ringer's acetate (Sterofundin, B Braun Medical). Patients were randomised in 26 ICUs in Denmark, Finland, Norway and Iceland between December 2009 and November 2011. All non-Danish hospitals were university hospitals and all ICUs were located at different hospitals. Exclusion criteria were: Age < 18 years, treatment with > 1000 ml of any synthetic colloid within the last 24 h prior to randomisation; any form of renal replacement therapy (RRT), severe hyperkalaemia (p-K > 6 mmol/l) within the last 6 h and acute burn injury $> 10\%$ of body surface area, previously randomised in the 6S trial, allergy towards HES or malic acid, liver or kidney transplantation during that hospitalization, intracranial bleeding within that hospitalization, enrolment into another ICU trial of drugs

with potential action on circulation, renal function or coagulation, and withdrawal of active therapy. Appropriate approvals and written consent from patients and/or legal substitutes were obtained according to national laws. The 6S trial was approved by the Danish Committee on Health Research Ethics. Patient information in the 6S trial database was anonymized and de-identified. No additional patient data were obtained for the present study and an ethics approval for the present study was not required according to Danish law. The protocol and the outcomes have been published [12–14].

In the present study we included all patients randomised into the 6S trial who had available fluid data from 24 hours before randomisation until day 3 post-randomisation, i.e. patients that had not died, had not been discharged and had no missing daily fluid data until day 3 after randomisation. As this was a post hoc study, a convenience sample was used and a sample size calculation was not performed.

The primary outcome measure was fluid resuscitation volume and the secondary outcome measure was total fluid input. Fluid resuscitation volume was defined as all masked and open-label trial fluids given in the first 3 days after randomisation in the 6S trial combined with crystalloids (not including fluids with medication) and colloids given from 24 hours before randomisation (day 0). Open label trial fluid (Ringer's acetate) was administered in case of the maximum dose of 33 ml/kg/24h masked trial fluid was exceeded. If a patient was withdrawn from the trial intervention, then all crystalloids (not including fluids with medication) and colloids given from time of withdrawal to the end of day 3 after randomisation were regarded as fluid resuscitation. Reasons for withdrawal were described in the published protocol [13]. Fluid data were registered daily in the 6S trial database with differentiation between trial fluids, crystalloids, colloids, glucose solutions, fluids with medication, nutrition, and blood products. Total fluid input was defined as the sum of all fluids, including the fluid resuscitation volume.

We decided *a priori* to include the following patient baseline characteristics in the multivariate analyses: Simplified Acute Physiology Score (SAPS II) [15], age, weight, highest lactate +/- 2 hours from randomisation, surgery performed prior to randomisation, allocation group (HES or Ringer's acetate) and individual Sequential Organ Failure Assessment (SOFA) subscores (cardiovascular, renal, liver, coagulation and respiratory) [16]. The CNS SOFA subscore was not registered in the 6S trial and was not included in the present study. The included hospital characteristics were Danish hospital (yes/no), university hospital (yes/no) and individual hospitals with at least 25 patients randomised in the 6S trial. The manuscript was prepared according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement [17].

Statistical analyses

We performed analyses using a generalised linear model with fluid volumes as outcome. The following model builds were used:

Model 1. Hospital characteristics:

Fluid volumes ~ Danish hospital (yes/no) + University hospital (yes/no) + patient baseline characteristics (in order to perform adjustment by trial site, trial sites with less than 25 randomised patients were combined into one site).

Model 2. Individual trial site (only trial sites with at least 25 randomised patients were included):

Fluid volumes ~ Trial site (as a factor) + patient baseline characteristics

Model 3. Patient baseline characteristics:

Fluid volumes ~ Patient baseline characteristics + Trial site (as factor where trial sites with less than 25 randomised patients were combined into one site in order to perform adjustment).

The patterns of residuals were evaluated for each analysis to ensure adequate goodness of fit. If > 5% of baseline values were missing, multiple imputations of the missing values were performed, which has been reported to reduce risk of bias [18]. For each missing baseline value 10 imputations were performed where the missing value was imputed from SAPS II, age, weight, highest lactate, surgery performed prior to randomisation, allocation (HES or Ringer's acetate) and individual SOFA component subscores, Danish hospital, university hospital, mortality within 90 days of randomisation and any use of RRT within 90 days of randomization. Complete case analyses were also performed.

Fluids given in the 24 hours prior to randomisation might include fluids given in the general ward where fluid registration was not as meticulous as in the ICU. Therefore, we conducted a sensitivity analysis excluding fluids given prior to randomisation, and another sensitivity analysis including the patients who had been discharged or had died within the first 3 days of randomisation. To investigate the impact of patients who were withdrawn from the intervention during the first 3 days after randomisation, we performed sensitivity analyses excluding these patients. We chose *a priori* to include trial site as a fixed-effect variable. To investigate the impact of this choice, we performed mixed model analyses with trial site analysed as a random-effects variable.

Statistical analyses were performed using SAS version 9.4 (SAS institute Inc., Cary, NC, USA). Two-sided P-values of less than 0.05 were considered statistically significant.

Results

Of the trial cohort of 798 patients, we included the 654 (82%) patients who had fluid volumes registered from day 0 to 3 (Fig 1). Of the 654 patients, 93 (14%) were withdrawed from the intervention during the first 3 days after randomisation resulting in 183/1962 (9%) of days where all administered crystalloids and colloids where included in the fluid resuscitation volume for the present analyses. Baseline characteristics are presented in Table 1.

Fluid volumes

The fluid resuscitation volume given from day 0 to day 3 was median 7,300 ml (interquartile range (IQR) 5,000–10,000 ml); total fluid input was median 16,912 ml (IQR 13,681–20,513 ml). Daily fluid volumes are presented in Fig 2. Fluids given with medication and nutrition combined constituted 72% of the difference between fluid resuscitation volume and total fluid input. The constituents daily of fluid input from day 0 to day 3 are presented in Table A in S1 Appendix.

Hospital characteristics and fluid volumes

Individual trial sites administered significantly different fluid resuscitation volumes and total fluid input adjusted by patient baseline characteristics ($p<0.001$ for both outcome measures–Fig 3). Being admitted at a university hospital was associated with decreased fluid resuscitation volume, but no statistically significant difference in total fluid input (Table 2). Being admitted to a Danish hospital was associated with increased total fluid input, but no statistically significant difference in fluid resuscitation volumes. Unadjusted analyses are presented in Table B in S1 Appendix.

Patient baseline characteristics and fluid volumes

In the multivariate generalised linear model, increased lactate, increased cardiovascular and renal SOFA subscores, decreased respiratory SOFA subscore and having surgery performed

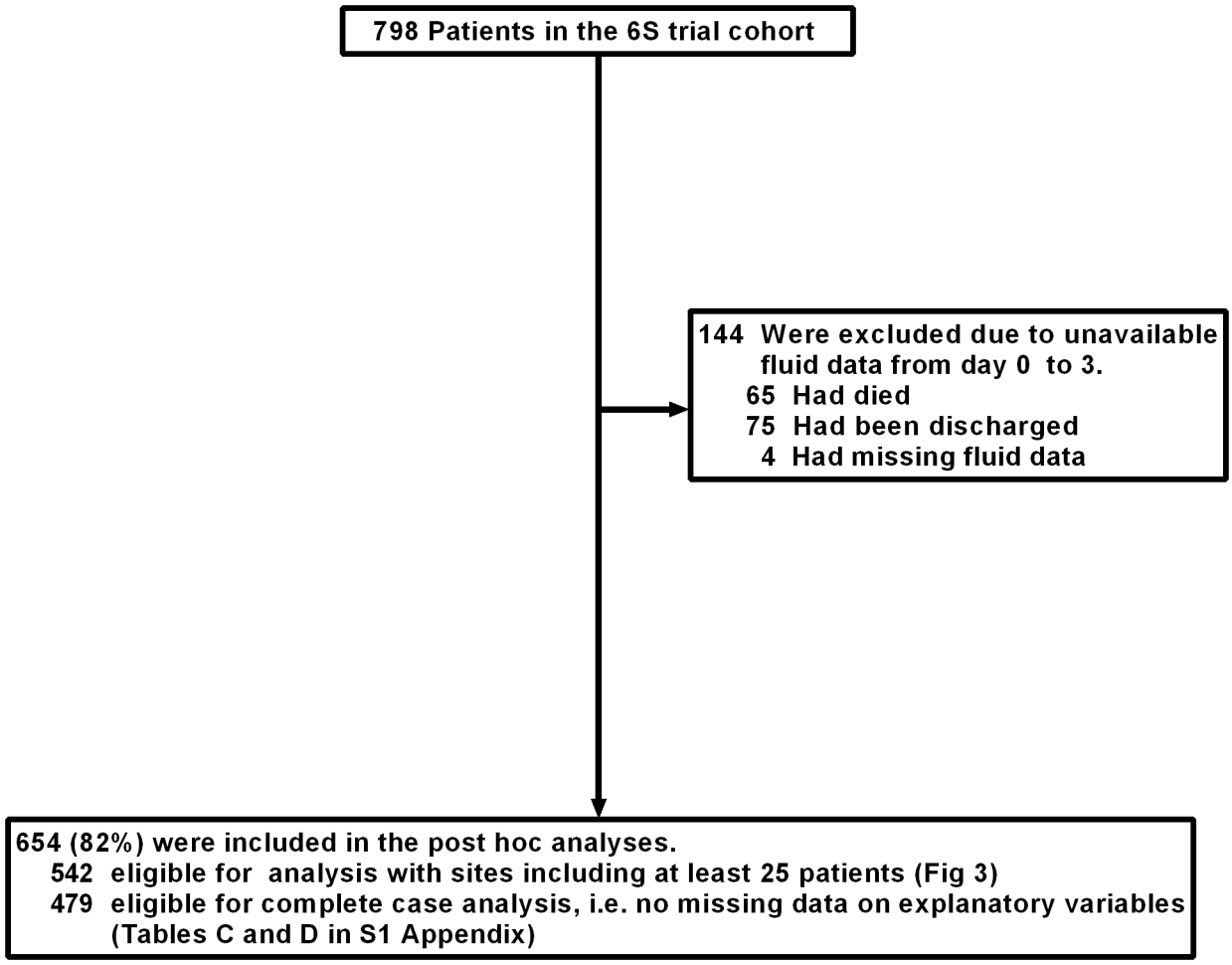

Fig 1. Flowchart of included patients.

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prior to randomisation were all independently associated with increased fluid resuscitation volume and total fluid input (Table 3). In addition weight and increased coagulation SOFA subscore were associated with increased fluid input. Unadjusted analyses are presented in Table B in S1 Appendix.

Missing data

Only the SAPS II (25% missing) needed multiple imputations. Complete case analyses did not change results noticeably (Tables C and D in S1 Appendix).

Sensitivity analyses

When excluding fluids given prior to randomisation there was no difference in fluid resuscitation volumes between university hospitals and non-university hospitals (Table E in S1 Appendix). Also, the cardiovascular SOFA subscore, the respiratory SOFA subscore and having surgery performed were no longer associated with differences in fluid resuscitation volume (Table F in S1 Appendix).

When including patients who had been discharged or had died within three days of randomisation, higher lactate and being admitted to a non-university hospital were not associated

Table 1. Patient baseline and hospital characteristics.

	Total cohort (n = 654)
Male	399 (61%)
Age (years)	66 (57–75)
SAPS II	50 (40–60)
Weight (kg)	78 (65–89)
Highest lactate +/- 2 hours from randomisation (mmol/l)	2.1 (1.4–3.4)
HES group	329 (50%)
Surgery prior to randomisation	232 (35%)
Source of sepsis	
- Lungs	369 (56%)
- Abdomen	209 (32%)
- Urinary tract	77 (12%)
- Soft tissue	74 (11%)
- Other source	59 (9%)
Hours from ICU admission to randomisation	4 (1–14)
SOFA score[1]	7 (5–9)
- Cardiovascular subscore	3 (1–4)
- Renal subscore	1 (0–2)
- Liver subscore	0 (0–1)
- Coagulation subscore	0 (0–1)
- Respiratory subscore	3 (2–3)
Danish hospital	583 (89%)
University hospital	321 (49%)
Randomised in trial site with $\geq$ 25 patients randomised (12 of 26 trial sites)	542 (83%)

All variables presented as median (interquartile range) or n (%). Individual SOFA subscores range from 0–4 with 4 being the most severe score.

[1] The CNS component of the SOFA score was not reported in the 6S trial and is not included in the analysis.

Abbreviations: HES, hydroxyethyl starch. ICU, intensive care unit. SOFA, Sequential Organ Failure Assessment.

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with increased fluid resuscitation volume with statistical significance in contrast to the primary analyses (Tables G and H in S1 Appendix).

The analyses with trial site as a random-effects variable (Tables I and J in S1 Appendix) and the sensitivity analyses excluding patients who were withdrawn from the intervention did not change the results noticeably (Tables K and L in S1 Appendix).

Discussion

In this post hoc analysis of data from the 6S trial we found that individual trial sites adjusted for patient baseline characteristics were associated with differences in administered fluid volumes in ICU patients with severe sepsis. Geographical differences in choice of fluid type have previously been reported [19], and the findings of the present study indicate differences in clinical practice not explained by patient characteristics and support our hypothesis that the vague recommendations on continued fluid therapy in sepsis result in differences in fluid therapy.

Patient baseline characteristics were associated with differences in fluid resuscitation volume. The association with fluid resuscitation volume differed between SOFA subscores. Thus,

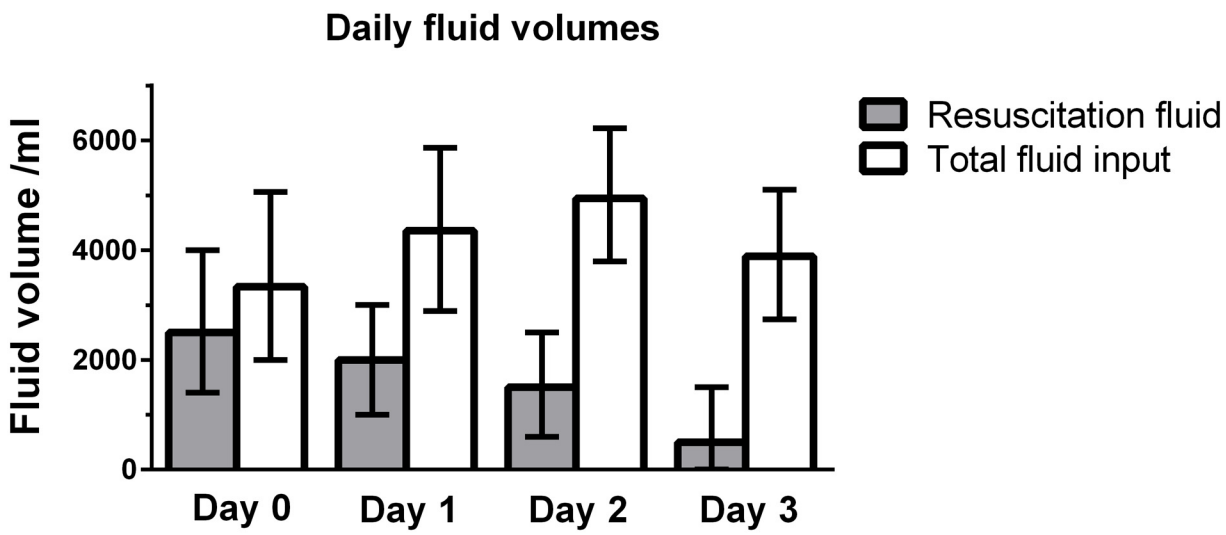

Fig 2. Daily fluid volumes presented as median with interquartile range (error bars). Resuscitation fluid was defined as crystalloids and colloids given from 24 hours prior to randomisation (day 0) until end of day 3 in the 6S trial.

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the association between combined SOFA score and fluid resuscitation volume may depend on the constitution of the SOFA subscores. In line with this, we found the SAPS II not to be statistically significantly associated with increased fluid resuscitation volume in either the adjusted or the unadjusted analysis. The SOFA score and the SAPS II are frequently used in multivariate analyses when adjusting for 'illness severity', but our data indicate that results must be interpreted with caution considering fluid volumes in sepsis. We unexpectedly found patient weight not to be statistically significantly associated with fluid resuscitation volume in either the multivariate or the univariate analysis, and the observed point estimate of 15 ml/kg difference was lower than expected. Recommendations for the initial fluid resuscitation are based on the baseline weight of the patient [11], and although the recommendations for continued fluid therapy do not explicitly mention weight, we had expected higher baseline weight to be associated with increased fluid resuscitation volume. From a physiological point of view it seems rational to increase fluid resuscitation volume with higher baseline weight due to the increased volume of distribution. Baseline weight, however, was associated with increased total fluid input.

Our finding that increased respiratory SOFA subscore was associated with less fluid resuscitation volume was in accordance with the results of the FACCT trial suggesting benefit of a conservative fluid strategy in patients with acute lung injury [20]. Our findings are also consistent with recent studies that found low blood pressure and increasing vasopressor dose (determinants of the cardiovascular SOFA subscore) to be the most frequent indications for fluid bolus in severe sepsis [21, 22]; oliguria (one of the determinants of renal SOFA subscore) was also a frequently used indication for fluid bolus in both studies. Of note, in the sensitivity analysis excluding fluids given prior to randomisation the cardiovascular SOFA subscore at baseline was not independently associated with increased fluid resuscitation volumes. Although these indications for fluid administration are frequent, it is still not established whether patients with severe sepsis and septic shock will benefit from receiving additional fluids. Alternative actions include continued/increased vasopressors or permitting wider physiological derangements [23]. The ability of treating clinicians to evaluate the balance between potential benefits and potential harms of fluid administration is impeded by the not yet fully understood hemodynamic consequences of giving fluids; a recent study found the relationship between cardiac

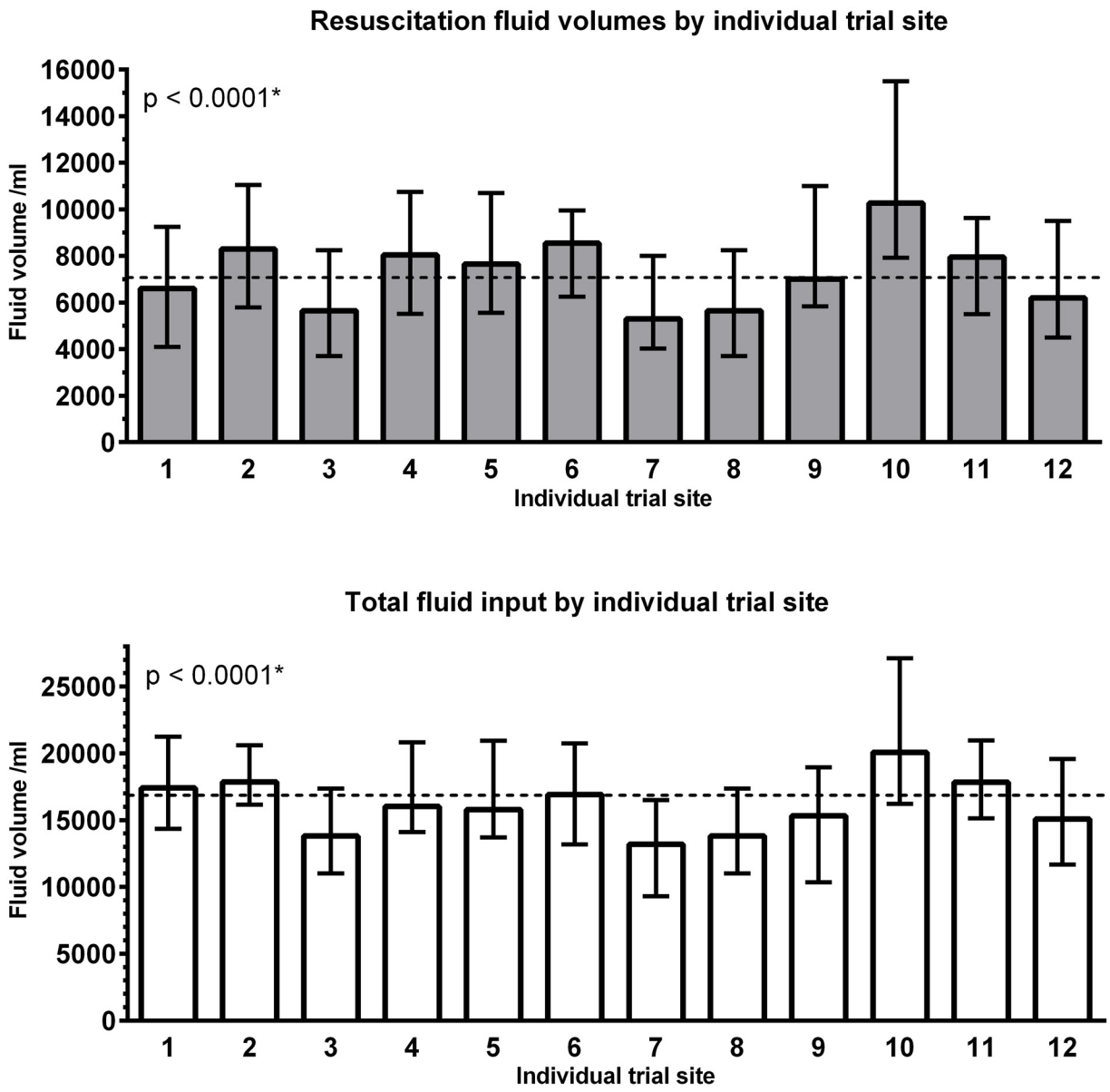

Fig 3. Resuscitation fluid volume (top panel) and total fluid input (bottom panel) by individual trial site with at least 25 randomised patients (n = 542). Fluid volumes presented as median with interquartile range (error bars). The horizontal dashed line denotes median for all patients. * P-value for trial site in a generalised linear model. Multiple generalised linear model build: Resuscitation fluid ~ Trial site (as a factor) + SAPS II + age + weight + highest lactate + surgery performed prior to randomisation (yes/no) + allocation (HES/Ringer's acetate) + cardiovascular SOFA subscore + renal SOFA subscore+ liver SOFA subscore + coagulation SOFA subscore + respiratory SOFA subscore.

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output and mean arterial pressure in response to a fluid bolus to be unpredictable and inconsistent [24].

There was a marked difference in the results between resuscitation fluid volume and total fluid input for Danish hospitals vs. non-Danish, where Danish hospitals had higher total fluid input, but no difference in fluid resuscitation volume. This result may indicate regional difference in use of other fluids (e.g. fluids with medication and nutrition) in addition to the differences in fluid resuscitation volumes already described. Excluding fluids given prior to

Table 2. Associations between hospital characteristics and fluid volumes given from day 0 to day 3. (n = 654).

	Resuscitation fluid (95% CI) (ml)	P-value	Total fluid input (95% CI) (ml)	P-value
Danish hospital vs. non-Danish hospital	-641 (-1787–506)	0.27	1773 (314–3232)	0.02
University hospital vs. non-university hospital	-825 (-1500– -150)	0.02	-618 (-1604–368)	0.22

Abbreviations: CI, confidence interval. HES, hydroxyethyl starch. SOFA, Sequential Organ Failure Assessment.

Multivariate generalised linear model build:

Fluid volume ~ Danish hospital (yes/no) + University hospital (yes/no) + SAPS II + age + weight + highest lactate + surgery performed prior to randomisation (yes/no) + allocation (HES/Ringer's acetate) + cardiovascular SOFA subscore + renal SOFA subscore+ liver SOFA subscore + SOFA subscore + coagulation + respiratory SOFA subscore

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randomisation noticeably changed the results for the cardiovascular SOFA subscore and surgery. These findings indicate that these patient baseline characteristics primarily affected the fluid resuscitation administered in the early phase of septic shock, whereas the strong association between higher lactate at baseline and increased fluid resuscitation volume persisted when excluding fluids given prior to randomisation.

The present study had several strengths. First, the 6S trial had relatively detailed daily registration of fluid input, which enabled us to differentiate between types of administered fluids. Fluids given with nutrition and medication constitute a large proportion of the fluid input during the first days of severe sepsis, and we were able to exclude these non-resuscitation fluids in our analyses. Second, the 6S trial randomised patients in different types of hospitals in four countries and thus increasing the external validity of our results. Third, the meticulous registration of fluid data resulted in only 0.4% of patients eligible for this study had missing fluid data.

There were also limitations. First, patients who had died or had been discharged were not included in the present analyses, which decrease the generalizability of the results. However,

Table 3. Associations between patient baseline characteristics and fluid volumes given from day 0 to day 3. (n = 654).

	Resuscitation fluids (95% CI) (ml)	P-value	Total fluid input (95% CI) (ml)	P-value
Age per year	-7 (-34–20)	0.60	-24 (-62–14)	0.21
SAPS II per unit	11 (-13–36)	0.35	14 (-24–51)	0.48
Weight per kg	15 (-0.3–31)	0.055	41 (18–64)	0.0005
Highest lactate per mmol/l	193 (53–332)	0.0069	289 (77–502)	0.008
Surgery vs. No surgery	1376 (732–2019)	< 0.0001	2941 (2006–3877)	< 0.0001
HES vs. Ringer's	-276 (-844–292)	0.34	305 (-518–1129)	0.47
SOFA subscores per unit				
- Cardiovascular subscore	375 (184–566)	0.0001	632 (340–924)	< 0.0001
- Renal subscore	269 (11–528)	0.04	353 (8–698)	0.045
- Coagulation subscore	63 (-232–357)	0.68	885 (454–1315)	< 0.0001
- Liver subscore	-43 (-460–374)	0.84	61 (-541–663)	0.84
- Respiratory subscore	-397 (-729 –-65)	0.02	-215 (-676–245)	0.36

Individual SOFA subscores range from 0–4 with 4 being the most severe score. CNS component of the SOFA score was not reported in the 6S trial and is not included in the analysis.

Abbreviations: CI, confidence interval. HES, hydroxyethyl starch. ICU, intensive care unit. SOFA, Sequential Organ Failure Assessment.

Multivariate generalised linear model build:

Fluid volume ~ SAPS II + age + weight + highest lactate + surgery performed prior to randomisation (yes/no) + allocation (HES/Ringer's acetate) + cardiovascular SOFA subscore + renal SOFA subscore+ liver SOFA subscore + coagulation SOFA subscore + respiratory SOFA subscore + trial site (as a factor, with hospital with less than 25 patients grouped)

doi:10.1371/journal.pone.0155767.t003

the sensitivity analysis including these patients did not change the results noticeably. Second, it is possible that hospitals participating in a randomised clinical trial of fluid therapy (in this case type of fluid) may have a different clinical practice as compared to non-participating hospitals in Scandinavia, thus introducing a potential selection bias. Third, all non-Danish hospitals were university hospitals which makes analyses of potential interaction unfeasible. Fourth, although the statistical assessments of model fits were adequate, there may be clinical considerations when interpreting the results. Especially the renal SOFA subscore should be interpreted with caution as extreme values (e.g. established anuria) might have led the clinicians to withhold further fluid resuscitation.

Conclusions

Hospital characteristics adjusted for patient baseline values were associated with differences in the administered fluid resuscitation volumes within the first 3 days of severe sepsis. Increased cardiovascular and renal SOFA subscores and decreased respiratory SOFA subscore at baseline were associated with increased fluid resuscitation volumes. Our data indicate variations in clinical practice not explained by patient characteristics emphasizing the need for RCTs of fluid resuscitation volumes in patients with severe sepsis.

Supporting Information

S1 Appendix. Supplemental tables and analyses.
(DOCX)

S1 Dataset. Dataset with multiple imputations.
(SAS7BDAT)

Acknowledgments

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Author Contributions

Conceived and designed the experiments: PBH NH JW AP. Performed the experiments: NH AP. Analyzed the data: PBH. Wrote the paper: PBH NH JW AP.

References

1. Lee WL, Slutsky AS. Sepsis and endothelial permeability. N Engl J Med. 2010; 363(7):689–91. doi: 10.1056/NEJMcibr1007320 PMID: 20818861
2. Suffredini AF, Fromm RE, Parker MM, Brenner M, Kovacs JA, Wesley RA, et al. The cardiovascular response of normal humans to the administration of endotoxin. N Engl J Med. 1989; 321(5):280–7. doi: 10.1056/NEJM198908033210503 PMID: 2664516
3. Aman J, van Nieuw Amerongen GP, Groeneveld ABJ. Revised Starling equation predicts pulmonary edema formation during fluid loading in the critically ill with presumed hypovolemia. Crit Care Med. 2012; 40:2742-.
4. Yunos NaM, Bellomo R, Hegarty C, Story D, Ho L, Bailey M. Association between a chloride-liberal vs chloride-restrictive intravenous fluid administration strategy and kidney injury in critically ill adults. JAMA. 2012; 308(15):1566–72. doi: 10.1001/jama.2012.13356 PMID: 23073953
5. Adrogué HJ, Madias NE. Aiding fluid prescription for the dysnatremias. Intensive Care Med. 1997; 23 (3):309–16. doi: 10.1007/s001340050333 PMID: 9083234
6. Rivers E, Nguyen B, Havstad S, Ressler J, Muzzin A, Knoblich B, et al. Early goal-directed therapy in the treatment of severe sepsis and septic shock. N Engl J Med. 2001; 345(19):1368–77. doi: 10.1056/NEJMoa010307 PMID: 11794169

7. Yealy DM, Kellum JA, Huang DT, Barnato AE, Weissfeld LA, Pike F, et al. A randomized trial of protocol-based care for early septic shock. N Engl J Med. 2014; 370(18):1683–93. doi: 10.1056/NEJMoa1401602 PMID: 24635773
8. Investigators A, Group ACT, Peake SL, Delaney A, Bailey M, Bellomo R, et al. Goal-directed resuscitation for patients with early septic shock. N Engl J Med. 2014; 371(16):1496–506. doi: 10.1056/NEJMoa1404380 PMID: 25272316
9. Mouncey PR, Osborn TM, Power GS, Harrison Da, Sadique MZ, Grieve RD, et al. Trial of early, goal-directed resuscitation for septic shock. N Engl J Med. 2015; 372(14):1301–11. doi: 10.1056/NEJMoa1500896 PMID: 25776532
10. Angus DC, Barnato aE, Bell D, Bellomo R, Chong CR, Coats TJ, et al. A systematic review and meta-analysis of early goal-directed therapy for septic shock: the ARISE, ProCESS and ProMISe Investigators. Intensive Care Med. 2015; 41(9):1549–60. doi: 10.1007/s00134-015-3822-1 PMID: 25952825
11. Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, et al. Surviving sepsis campaign: International guidelines for management of severe sepsis and septic shock, 2012. Intensive Care Med. 2013; 39(2):165–228. doi: 10.1007/s00134-012-2769-8 PMID: 23361625
12. Perner A, Haase N, Guttormsen AB, Tenhunen J, Klemenzson G, Åneman A, et al. Hydroxyethyl Starch 130/0.42 versus Ringer's Acetate in Severe Sepsis. N Engl J Med. 2012; 367(2):124–34. doi: 10.1056/NEJMoa1204242 PMID: 22738085
13. Perner A, Haase N, Wetterslev J, Aneman A, Tenhunen J, Guttormsen AB, 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. Trials. 2011; 12(1):24-.
14. Perner A, Haase N, Winkel P, Guttormsen AB, Tenhunen J, Klemenzson G, et al. Long-term outcomes in patients with severe sepsis randomised to resuscitation with hydroxyethyl starch 130/0.42 or Ringer's acetate. Intensive Care Med. 2014; 40(7):927–34. Epub 2014/05/09. doi: 10.1007/s00134-014-3311-y PMID: 24807084.
15. Le Gall JR, Lemeshow S, Saulnier F. A new Simplified Acute Physiology Score (SAPS II) based on a European/North American multicenter study. JAMA. 1993; 270(24):2957–63. PMID: 8254858
16. Vincent JL, Moreno R, Takala J, Willatts S, De Mendonça A, Bruining H, et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. Intensive Care Med. 1996; 22(7):707–10.
17. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. J Clin Epidemiol. 2008; 61(4):344–9. doi: 10.1016/j.jclinepi.2007.11.008 PMID: 18313558
18. Crowe BJ, Lipkovich IA, Wang O. Comparison of several imputation methods for missing baseline data in propensity scores analysis of binary outcome. Pharm Stat. 2010; 9(4):269–79. doi: 10.1002/pst.389 PMID: 19718652
19. Finfer S, Liu B, Taylor C, Bellomo R, Billot L, Cook D, et al. Resuscitation fluid use in critically ill adults: an international cross-sectional study in 391 intensive care units. Crit Care. 2010; 14(5):R185–R. doi: 10.1186/cc9293 PMID: 20950434
20. Wiedemann HP, Wheeler AP, Bernard GR, Thompson BT, Hayden D, DeBoisblanc B, et al. Comparison of two fluid-management strategies in acute lung injury. The New England journal of medicine. 2006; 354(24):2564–75. doi: 10.1056/NEJMoa062200 PMID: 16714767
21. Bihari S, Baldwin CE, Bersten AD, Ou J, Holt AW, Prakash S, et al. Post resusicitation fluid boluses in severe sepsis or septic shock: prevalence and efficacy (price study). Crit Care Resusc. 2013; 12 (1):66–70.
22. Cecconi M, Hofer C, Teboul J-L, Pettila V, Wilkman E, Molnar Z, et al. Fluid challenges in intensive care: the FENICE study: A global inception cohort study. Intensive Care Med. 2015; 41(9):1529–37.
23. Perner A, Vieillard-Baron A, Bakker J. Fluid resuscitation in ICU patients: quo vadis? Intensive Care Med. 2015; 41(9):1667–9. doi: 10.1007/s00134-015-3900-4 PMID: 26072659
24. Monge García MI, Guijo González P, Gracia Romero M, Gil Cano A, Oscier C, Rhodes A, et al. Effects of fluid administration on arterial load in septic shock patients. Intensive Care Med. 2015; 41(7):1247–55. doi: 10.1007/s00134-015-3898-7 PMID: 26077088

Supplement

Table A. Constituents of the total fluid input from 24 hours prior to randomisation until end of day 3. (n=654)

	Fluid volumes, ml Median (IQR) [mean]
24 hours prior to randomisation	
Crystalloids	2000 (1000-3700) [2640]
Synthetic colloids	0 (0-500) [312]
Albumin	0 (0-0) [52]
Blood products (RBC, FFP, platelets)	0 (0-0) [285]
Other fluids (incl. fluids with medication and nutrition)	0 (0-868) [551]
Day 1	
Trial fluid + open-label trial fluid	1700 (1000-2850) [2051]
Crystalloids	200 (0-1000) [768]
Synthetic colloids	0 (0-0) [22]
Albumin	0 (0-0) [11]
Blood products (RBC, FFP, platelets)	0 (0-0) [207]
Nutrition	300 (0-900) [508]
Fluids with medication	700 (321-1147) [806]
Other fluids (incl. water and glucose solutions)	0 (0-180) [213]
Day 2	
Trial fluid + open-label trial fluid	1500 (500-2500) [1662]
Crystalloids	0 (0-500) [455]
Synthetic colloids	0 (0-0) [9]
Albumin	0 (0-0) [14]
Blood products (RBC, FFP, platelets)	0 (0-245) [258]
Nutrition	1193 (716-1600) [1168]
Fluids with medication	1167 (742-1688) [1256]
Other fluids (incl. water and glucose solutions)	0 (0-510) [386]
Day 3	
Trial fluid + open-label trial fluid	500 (0-1100) [773]
Crystalloids	0 (0-290) [333]
Synthetic colloids	0 (0-0) [5]
Albumin	0 (0-0) [12]
Blood products (RBC, FFP, platelets)	0 (0-0) [169]
Nutrition	1356 (718-1767) [1268]
Fluids with medication	967 (515-1585) [1099]
Other fluids (incl. water and glucose solutions)	0 (0-440) [319]

Abbreviations: RBC, red blood cells; FFP, fresh frozen plasma

Unadjusted analyses

Table B. Univariate analyses of associations between patient baseline characteristics and fluid resuscitation volumes given from day 0 to day 3. (n=654)

	Resuscitation fluid (95% CI) / ml	P-value (univariate)
Age	0.2 (-23 – 23)	0.99
SAPS II	14 (-5 – 33)	0.15
Weight	16 (-1 – 32)	0.071
Highest lactate	235 (105 – 364)	0.0004
Surgery vs. No surgery	1175 (538 – 1812)	0.0003
HES vs. Ringer's	-311 (-926 – 304)	0.32
SOFA subscores		
- Cardiovascular subscore	408 (207 – 609)	<0.0001
- Renal subscore	414 (183 – 646)	0.0005
- Coagulation subscore	46 (-223 – 314)	0.74
- Liver subscore	103 (-298 – 504)	0.62
- Respiratory subscore	-370 (-683 – -57)	0.021
Danish hospital	-681 (-1668 – 307)	0.18
University hospital	-391 (-1006 – 224)	0.21
Individual trial sites[1]	NA	<0.0001

[1] Only trial sites with at least 25 randomised patients included in the analysis (n=542). Individual SOFA subscores range from 0-4 with 4 being the most severe score. CNS component of the SOFA score was not reported in the 6S trial and not included in the analysis. Abbreviations: HES, hydroxyethyl starch. ICU, intensive care unit. SOFA, Sequential Organ Failure Assessment.

Complete case analyses

Table C. Complete case analyses. Multivariate analyses of associations between hospital characteristics and fluid resuscitation volumes given from day 0 to day 3 adjusted for patient baseline characteristics. (n=479)

	Resuscitation fluid (95% CI) / ml	P-value
Model A:		
Danish hospital	-563 (-1840 – 714)	0.39
University hospital	-1003 (-1815 – -192)	0.02
Model B:		
Individual trial sites[1]	NA	<0.0001

[1]Only trial sites with at least 25 patients randomised included (n=397 in 12 trial sites).
Abbreviations: CI, confidence interval. NA, not applicable.
Multivariate generalised linear model A build:
Resuscitation fluid ~ Danish hospital (yes/no) + University hospital (yes/no) + patient baseline characteristics
Multiple generalised linear model B build:
Resuscitation fluid ~ Trial site (as a factor) + patient baseline characteristics

Table D. Complete case analysis. Multivariate analysis of associations between patient baseline characteristics and fluid resuscitation volumes given from day 0 to day 3. (n=479)

	Resuscitation fluid (95% CI) / ml	P-value[1]
Age	-11 (-40 – 18)	0.45
SAPS II	18 (-10 – 46)	0.21
Weight	20 (1 – 38)	0.042
Highest lactate	187 (27 – 346)	0.02
Surgery vs. No surgery	1372 (597 – 2147)	0.0005
HES vs. Ringer's	-151 (-844 – 541)	0.67
SOFA subscores		
- Cardiovascular subscore	392 (134 – 651)	0.003
- Renal subscore	286 (-5 – 577)	0.054
- Coagulation subscore	73 (-246 – 392)	0.65
- Liver subscore	70 (-412 – 552)	0.78
- Respiratory subscore	-618 (-1005 – -230)	0.002

Individual SOFA subscores range from 0-4 with 4 being the most severe score. CNS component of the SOFA score was not reported in the 6S trial and is not included in the analysis.
Abbreviations: CI, confidence interval. HES, hydroxyethyl starch. ICU, intensive care unit. SOFA, Sequential Organ Failure Assessment.
Multivariate generalised linear model build:
Resuscitation fluid ~ SAPS II + age + weight + highest lactate + surgery performed prior to randomisation (yes/no) + allocation (HES/Ringer's acetate) + cardiovascular SOFA subscore + renal SOFA subscore+ liver SOFA subscore + SOFA subscore + coagulation + respiratory SOFA subscore + trial site (as a factor, with hospital with less than 25 patients grouped)

Fluids given after randomisation only

Table E. Multivariate analyses of associations between hospital characteristics and fluid resuscitation volumes given from day 1 to day 3 adjusted for patient baseline characteristics. (n=654)

	Resuscitation fluids (95% CI) / ml	P-value
Model A:		
Danish hospital	566 (-444 – 1577)	0.27
University hospital	-264 (-758 – 230)	0.29
Model B:		
Individual trial sites[1]	NA	0.001

[1]Only trial sites with at least 25 patients randomised included (n=542 in 12 trial sites).
Abbreviations: CI, confidence interval. NA, not applicable.
Multivariate generalised linear model A build:
Resuscitation fluid ~ Danish hospital (yes/no) + University hospital (yes/no) + patient baseline characteristics
Multiple generalised linear model B build:
Resuscitation fluid ~ Trial site (as a factor) + patient baseline characteristics

Table F. Multivariate linear regression analysis of associations between patient baseline characteristics and fluid resuscitation volumes given from day 1 to day 3 adjusted for patient baseline characteristics. (n=654)

	Resuscitation fluids (95% CI) / ml	P-value
Age	1 (-20 – 22)	0.92
SAPS II	12 (-6 – 31)	0.19
Weight	7 (-5 – 20)	0.24
Highest lactate	173 (53 – 294)	0.005
Surgery vs. No surgery	450 (-73 – 973)	0.09
HES vs. Ringer's	-421 (-866 – 24)	0.06
SOFA subscores		
- Cardiovascular subscore	18 (-130 – 167)	0.81
- Renal subscore	212 (9 – 415)	0.041
- Coagulation subscore	-155 (-358 – 47)	0.13
- Liver subscore	12 (-318 – 340)	0.95
- Respiratory subscore	-219 (-483 – 46)	0.10

Individual SOFA subscores range from 0-4 with 4 being the most severe score. CNS component of the SOFA score was not reported in the 6S trial and is not included in the analysis.
Abbreviations: CI, confidence interval. HES, hydroxyethyl starch. ICU, intensive care unit. SOFA, Sequential Organ Failure Assessment.
Multivariate generalised linear model build:
Resuscitation fluid ~ SAPS II + age + weight + highest lactate + surgery performed prior to randomisation (yes/no) + allocation (HES/Ringer's acetate) + cardiovascular SOFA subscore + renal SOFA subscore+ liver SOFA subscore + SOFA subscore + coagulation + respiratory SOFA subscore + trial site (as a factor, with hospital with less than 25 patients grouped)

Discharged and deceased patients included

Table G. Multivariate analyses of associations between hospital characteristics and fluid resuscitation volumes given from day 0 to day 3 adjusted for patient baseline characteristics. Patients who had died or had been discharge within the first 3 days after randomisation included in the analysis. (n=794)

	Resuscitation fluids (95% CI) / ml	P-value
Model A:		
Danish hospital	-542 (-1573 – 488)	0.30
University hospital	-552 (-1166 – 63)	0.08
Model B:		
Individual trial sites[1]	NA	<0.0001

[1]Only trial sites with at least 25 patients randomised included (n=662 in 12 trial sites).
Abbreviations: CI, confidence interval. NA, not applicable.
Multivariate generalised linear model A build:
Resuscitation fluid ~ Danish hospital (yes/no) + University hospital (yes/no) + patient baseline characteristics
Multiple generalised linear model B build:
Resuscitation fluid ~ Trial site (as a factor) + patient baseline characteristics

Table H. Multivariate linear regression analysis of associations between patient baseline characteristics and fluid resuscitation volumes given from day 0 to day 3 adjusted for patient baseline characteristics. Patients who had died or had been discharge within the first 3 days after randomisation included in the analysis. (n=794)

	Resuscitation fluids (95% CI) / ml	P-value[1]
Age	-5 (-29 – 19)	0.66
SAPS II	7 (-17 – 31)	0.54
Weight	13 (-0.5 – 27)	0.058
Highest lactate	75 (-21 – 171)	0.13
Surgery vs. No surgery	1209 (619 – 1798)	<0.0001
HES vs. Ringer's	-250 (-769 – 269)	0.35
SOFA subscores		
- Cardiovascular subscore	383 (204 – 563)	<0.0001
- Renal subscore	261 (28 – 495)	0.03
- Coagulation subscore	65 (-204 – 334)	0.63
- Liver subscore	-88 (-452 – 276)	0.64
- Respiratory subscore	-327 (-623 – -30)	0.03

Individual SOFA subscores range from 0-4 with 4 being the most severe score. CNS component of the SOFA score was not reported in the 6S trial and is not included in the analysis.
Abbreviations: CI, confidence interval. HES, hydroxyethyl starch. ICU, intensive care unit. SOFA, Sequential Organ Failure Assessment.
Multivariate generalised linear model build:
Resuscitation fluid ~ SAPS II + age + weight + highest lactate + surgery performed prior to randomisation (yes/no) + allocation (HES/Ringer's acetate) + cardiovascular SOFA subscore + renal SOFA subscore+ liver SOFA subscore + SOFA subscore + coagulation + respiratory SOFA subscore + trial site (as a factor, with hospital with less than 25 patients grouped)

Performing analyses using mixed model with trial site as a random effect

Table I. Mixed model analysis of associations between individual trial sites and fluid resuscitation volumes given from day 0 to day 3 adjusted for patient baseline characteristics. (n=542)

	Resuscitation fluids (95% CI) / ml	P-value
Individual trial sites[1]	NA	0.03

[1]Only trial sites with at least 25 patients randomised included (n=542 in 12 trial sites).
Abbreviations: CI, confidence interval. NA, not applicable.
Mixed model:
Resuscitation fluid ~ Trial site (as a random-effects factor) + patient baseline characteristics

Table J. Mixed model analysis of associations between patient baseline characteristics and fluid resuscitation volumes given from day 0 to day 3 adjusted for patient baseline characteristics. (n=654)

	Resuscitation fluids (95% CI) / ml	P-value[1]
Age	-7 (-31 – 17)	0.58
SAPS II	11 (-14 – 35)	0.40
Weight	14 (-2 – 31)	0.08
Highest lactate	191 (60 – 322)	0.004
Surgery vs. No surgery	1389 (742 – 2036)	<0.0001
HES vs. Ringer's	-275 (-851 – 302)	0.35
SOFA subscores		
- Cardiovascular subscore	379 (166 – 593)	0.0005
- Renal subscore	269 (25 – 513)	0.03
- Coagulation subscore	73 (-200 – 347)	0.60
- Liver subscore	-49 (-440 – 342)	0.81
- Respiratory subscore	-395 (-714 – -76)	0.02

Individual SOFA subscores range from 0-4 with 4 being the most severe score. CNS component of the SOFA score was not reported in the 6S trial and is not included in the analysis.
Abbreviations: CI, confidence interval. HES, hydroxyethyl starch. ICU, intensive care unit. SOFA, Sequential Organ Failure Assessment.
Multivariate generalised linear model build:
Resuscitation fluid ~ SAPS II + age + weight + highest lactate + surgery performed prior to randomisation (yes/no) + allocation (HES/Ringer's acetate) + cardiovascular SOFA subscore + renal SOFA subscore+ liver SOFA subscore + SOFA subscore + coagulation + respiratory SOFA subscore + trial site (as a random-effects factor, with hospital with less than 25 patients grouped)

Excluding patients who were withdrawn from the intervention during the first 3 days after randomisation

Table K. Multivariate analyses of associations between hospital characteristics and fluid resuscitation volumes given from day 0 to day 3 adjusted for patient baseline characteristics. Patients who were withdrawn from the intervention during the first 3 days were excluded (n=561).

	Resuscitation fluids (95% CI) / ml	P-value
Model A:		
Danish hospital	-427 (-1612 – 758)	0.48
University hospital	-807 (-1488 – -126)	0.02
Model B:		
Individual trial sites[1]	NA	<0.0001

[1]Only trial sites with at least 25 patients randomised included (n=465 in 12 trial sites).
Abbreviations: CI, confidence interval. NA, not applicable.
Multivariate generalised linear model A build:
Resuscitation fluid ~ Danish hospital (yes/no) + University hospital (yes/no) + patient baseline characteristics
Multiple generalised linear model B build:
Resuscitation fluid ~ Trial site (as a factor) + patient baseline characteristics

Table L. Multivariate analysis of associations between patient baseline characteristics and fluid resuscitation volumes given from day 0 to day 3 adjusted for patient baseline characteristics. Patients who were withdrawn from the intervention during the first 3 days were excluded (n=561).

	Resuscitation fluids (95% CI) / ml	P-value[1]
Age	3 (-23 – 29)	0.84
SAPS II	14 (-12 – 41)	0.28
Weight	15 (-2 – 31)	0.08
Highest lactate	212 (60 – 364)	0.006
Surgery vs. No surgery	1300 (624 – 1976)	0.0002
HES vs. Ringer's	-181 (-769 – 406)	0.55
SOFA subscores		
- Cardiovascular subscore	420 (225 – 615)	<0.0001
- Renal subscore	265 (-10 – 541)	0.06
- Coagulation subscore	-107 (-417 – 202)	0.50
- Liver subscore	-95 (-536 – 345)	0.67
- Respiratory subscore	-491 (-847 – -135)	0.007

Individual SOFA subscores range from 0-4 with 4 being the most severe score. CNS component of the SOFA score was not reported in the 6S trial and is not included in the analysis.
Abbreviations: CI, confidence interval. HES, hydroxyethyl starch. ICU, intensive care unit. SOFA, Sequential Organ Failure Assessment.
Multivariate generalised linear model build:
Resuscitation fluid ~ SAPS II + age + weight + highest lactate + surgery performed prior to randomisation (yes/no) + allocation (HES/Ringer's acetate) + cardiovascular SOFA subscore + renal SOFA subscore+ liver SOFA subscore + SOFA subscore + coagulation + respiratory SOFA subscore + trial site (as a factor, with hospital with less than 25 patients grouped)

PAPER II

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SEVEN-DAY PROFILE PUBLICATION

Restricting volumes of resuscitation fluid in adults with septic shock after initial management: the CLASSIC randomised, parallel-group, multicentre feasibility trial

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Abstract

Purpose: We assessed the effects of a protocol restricting resuscitation fluid vs. a standard care protocol after initial resuscitation in intensive care unit (ICU) patients with septic shock.

Methods: We randomised 151 adult patients with septic shock who had received initial fluid resuscitation in nine Scandinavian ICUs. In the fluid restriction group fluid boluses were permitted only if signs of severe hypoperfusion occurred, while in the standard care group fluid boluses were permitted as long as circulation continued to improve.

Results: The co-primary outcome measures, resuscitation fluid volumes at day 5 and during ICU stay, were lower in the fluid restriction group than in the standard care group [mean differences −1.2 L (95 % confidence interval −2.0 to −0.4); $p < 0.001$ and −1.4 L (−2.4 to −0.4) respectively; $p < 0.001$]. Neither total fluid inputs and balances nor serious adverse reactions differed statistically significantly between the groups. Major protocol violations occurred in 27/75 patients in the fluid restriction group. Ischaemic events occurred in 3/75 in the fluid restriction group vs. 9/76 in the standard care group (odds ratio 0.32; 0.08–1.27; $p = 0.11$), worsening of acute kidney injury in 27/73 vs. 39/72 (0.46; 0.23–0.92; $p = 0.03$), and death by 90 days in 25/75 vs. 31/76 (0.71; 0.36–1.40; $p = 0.32$).

Conclusions: A protocol restricting resuscitation fluid successfully reduced volumes of resuscitation fluid compared with a standard care protocol in adult ICU patients with septic shock. The patient-centred outcomes all pointed towards benefit with fluid restriction, but our trial was not powered to show differences in these exploratory outcomes.

Trial registration: NCT02079402.

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The members of the CLASSIC Trial Group are listed in the Acknowledgments.

Take-home message: A fluid restriction protocol in septic shock resulted in less resuscitation fluid being given to fewer patients. The patient-centred outcomes all pointed towards benefit with fluid restriction, but our trial was not powered to show differences in these exploratory outcomes.

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Keywords: Critical care, Fluid therapy, Intensive care, Resuscitation, Sepsis, Septic, Shock

Introduction

Fluid resuscitation is the mainstay of cardiovascular interventions for patients with septic shock [1]. Intravenous fluid may improve the circulation and organ perfusion by increasing cardiac output, but may also be associated with harmful effects through peripheral and organ oedema. The exact physiology of fluid resuscitation and the relation to patient-centred outcomes are, however, not yet fully elucidated.

In the clinical practice guideline for adults with septic shock, it is recommended to give a minimum of 30 mL/kg of crystalloid solutions during initial resuscitation and to continue to give fluids as long as the circulation improves [1]. However, there are limited high-quality data supporting these recommendations [1]; increased cumulative fluid balances at 12 h and 4 days have been associated with increased mortality in adult patients with septic shock [2] and, similarly, increased daily fluid balances from day 2 until day 7 have been associated with increased mortality in septic shock in adjusted analyses [3]. In addition, a large randomised trial showed increased mortality in febrile African children with circulatory impairment who received fluid boluses in addition to maintenance fluid as compared to those who received maintenance alone [4].

Taken together, current guidelines on volumes of resuscitation fluid in septic shock are based on low-quality evidence, and it is possible that higher fluid volumes may harm these patients. Therefore, we designed the Conservative vs. Liberal Approach to fluid therapy of Septic Shock in Intensive Care (CLASSIC) trial with the objective to assess the feasibility and effects of a protocol restricting resuscitation fluid after initial resuscitation on fluid volumes and balances and explorative outcome measures in intensive care unit (ICU) patients with septic shock. We focused on volumes of resuscitation fluid, rather than total fluid inputs or fluid balances, because resuscitation fluid is given with the specific aim to improve the circulation. Resuscitation fluid is, therefore, likely to have a different balance between benefit and harm than that of fluids given as maintenance or with nutrition and medications.

Methods

Trial design and conduct

The management committee wrote the trial protocol, which was approved by the Medicines Agency, Ethics Committee and Data Protection Agency in Denmark and the Ethics Committee in Helsinki, Finland. The protocol and the statistical analysis plan, which were written before closing the trial database, are provided in the Electronic Supplementary Material (ESM) 2. The trial was registered at http://www.clinicaltrials.gov (number NCT02079402) before enrolment of the first patient and conducted according to Good Clinical Practice (EU Directive 2001/20) including monitoring of consents and source data by external staff.

The CLASSIC trial was an investigator-initiated, multicentre, stratified (by site because these may influence volumes of resuscitation fluid [5]), parallel-group clinical trial with adequate computer generation of the allocation sequence with permuted blocks of varying sizes of 2 or 4 and allocation concealment by a Web-based, centralised randomisation system. We randomised patients with septic shock in nine general ICUs 1:1 to restrictive fluid resuscitation or standard care. The allocation was blinded for the statistician.

In Denmark, informed consent was obtained from two physicians who were independent of the trial prior to randomisation. In Finland, deferred consent was used. In all cases informed consent was obtained from the next of kin and the patient as soon as possible after randomisation. If consent was withdrawn or not granted, permission was asked for continued registration and use of data.

Patients

We screened patients aged 18 years or above (1) who were in the ICU, (2) who fulfilled the criteria for sepsis within the previous 24 h, (3) who had suspected or confirmed severe circulatory impairment—defined as systolic blood pressure below 90 mmHg, heart rate above 140 beats/min, lactate at least 4 mmol/L, or use of vasopressors—for no more than 12 h including the hours preceding ICU admission, (4) who had received at least 30 mL/kg ideal body weight (IBW) of fluid in the last 6 h, and (5) who had shock defined as ongoing infusion of norepinephrine to maintain blood pressure (the detailed trial definitions, including those regarding a change during trial in the definition of criterion 3, are provided in ESM 1 and ESM 2). Patients were excluded for the reasons shown in Fig. 1.

Interventions

In both intervention groups, use of resuscitation fluid was per protocol and mean arterial pressure (MAP) of at least 65 mmHg (or a target decided by the clinicians) was maintained by the use of continuous infusion of norepinephrine. The choice of crystalloid solutions was at the discretion of the treating clinicians, but the use of colloid solutions for resuscitation was regarded as a protocol violation to alleviate the risk of differences in the type of fluid administered between the intervention groups. Suggestions for the use of selected co-interventions were

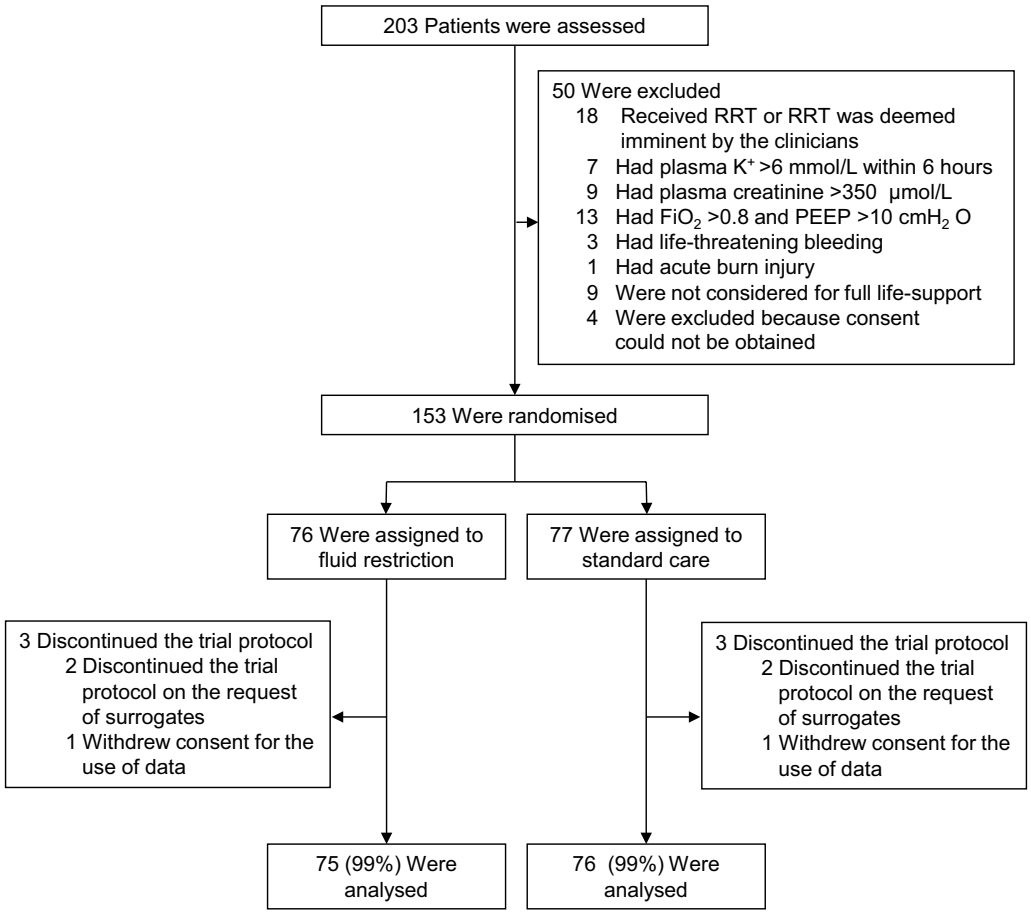

Fig. 1 Flow of trial participants in the CLASSIC trial. Patients with septic shock assessed, excluded, randomised and followed up in the CLASSIC trial. Twelve patients fulfilled 2 or 3 exclusion criteria. Two patients were excluded post-randomisation in the recruitment period because they withdrew consent for the use of data. Two additional patients were randomised to obtain the full sample size of 150 patients. One additional patient was randomised within an hour of the randomisation of patient no. 150 before the Web-based randomisation portal was closed. *RRT* renal replacement therapy, *PEEP* positive end-expiratory pressure

provided, including fluid therapy for other indications than resuscitation (ESM 1); substitution of overt fluid loss was allowed in both groups.

In the fluid restriction group, isotonic crystalloid (saline or Ringer's solutions) fluid boluses of 250–500 mL could be given intravenously during ICU stay in the case of severe hypoperfusion defined as either (1) plasma concentration of lactate of at least 4 mmol/L, (2) MAP below 50 mmHg in spite of the infusion of norepinephrine, (3) mottling beyond the edge of the kneecap (mottling score greater than 2) [6], or (4) oliguria, but only in the first 2 h after randomisation, defined as urinary output at most 0.1 mL/kg IBW in the last hour. The cut-off value of lactate was chosen on the basis of Surviving Sepsis Campaign (SSC) guidelines [1] and data indicating that a marked increase in mortality occurs at lactate values above 4 mM [7]. Fulfilment of at least one of these criteria was a prerequisite for administration of a fluid bolus, but administration was not mandated. The effect of a fluid bolus was to be assessed by re-evaluation of the four hypoperfusion criteria mentioned above before a repeated fluid bolus or after 30 min at the latest.

In the standard care group, isotonic crystalloid (saline or Ringer's solutions) fluid boluses could be given intravenously during ICU stay as long as haemodynamic variables improved including dynamic (e.g. stroke volume variation) or static (e.g. blood pressure, heart rate) variable(s) of the clinician's choice as outlined in the SSC guideline [1]. The effect of a fluid bolus was to be assessed by re-evaluation before a repeated fluid bolus or after 30 min at the latest.

Outcome measures

The co-primary outcomes were the amount of resuscitation fluid (defined as the cumulated volumes of 0.9 % saline,

Ringer's lactate, Ringer's acetate and colloid solutions given in the ICU for circulatory impairment as noted by the clinicians) in the first 5 days after randomisation and the amount of resuscitation fluid given after randomisation during the entire ICU stay. The latter was promoted from a secondary outcome to a co-primary outcome during the trial so that the full intervention period was reflected in the primary outcome. This change was done before the data were available for analyses. The details about this protocol change and the full definitions of all outcomes are provided in ESM 1 and in the trial protocol (ESM 2).

The secondary outcome measures were total fluid input given in the ICU at day 5 after randomisation and during the entire ICU stay, fluid balance in ICU at day 5 after randomisation and for entire ICU stay, number of patients with violations of the fluid resuscitation protocol, and rates of serious adverse reactions for isotonic crystalloids or norepinephrine in the ICU.

Exploratory outcomes were death within 90 days after randomisation, time to death with censoring 90 days after the last patient had been randomised, days alive without the use of mechanical ventilation or renal replacement therapy in the 90-day period, the number of patients with ischaemic events during the ICU stay, maximum change in plasma creatinine during the ICU stay, and number of patients with worsening of acute kidney injury (AKI) according to the KDIGO criteria [8] (values of plasma creatinine were assessed in ICU and the use of renal replacement therapy in the 90 days after randomisation; the urinary output criteria were not assessed). For patients without AKI at baseline, development of AKI after randomisation was regarded as worsening of AKI.

Statistical analysis

One hundred and fifty patients were needed to show a 1.7-L difference in volumes of resuscitation fluid within the first 5 days between the groups on the basis of the mean volume of resuscitation fluid observed in the 6S trial [5.3 L (standard deviation 3.7 L)] [9], an alpha of 5 % (two-sided) and a power of 80 %. The implications for the sample size estimation of the change from one to two co-primary outcomes are provided in the statistical analysis plan in the trial protocol (ESM 2).

In the recruitment period we excluded two patients after randomisation because they withdrew consent for the use of data. We randomised two additional patients to obtain the full sample size. One additional patient was randomised within an hour of patient no. 150 before the randomisation portal was closed (Fig. 1).

The statistician (P.W.) performed all the analyses blinded for the intervention and according to the ICH-GCP guidelines E9 [10] and the statistical analysis plan, in which the handling of missing data is also described (ESM 2). We performed the analyses in the intention-to-treat population defined as all randomised patients except those who withdrew consent for the use of data. We defined the per-protocol population as all patients in the intention-to-treat population except those who had a protocol violation (Table S1 in ESM 1).

In the primary analyses, we compared data in the two groups by the non-parametric van Elteren test or the general linear model for ordinal and rate data adjusted for the stratification variable (trial site) [10], logistic regression analysis for binary outcome measures adjusted for site and by logrank test and Cox analysis (adjusted for site) for time to death. Sites including less than 10 patients were grouped in the adjusted analyses. We also compared the co-primary outcomes in an analysis adjusted for predefined risk factors at baseline (age, weight, norepinephrine dose at randomisation, surgery prior to randomization and more than 5 L of fluid given prior to randomization), in the per-protocol population and in the predefined subgroup analysis of patients who had received more than 5 L of fluid (crystalloids, colloids, and blood products) in the 24-h prior to randomisation. We performed all analyses using SAS software, version 9.3, and SPSS software, version 17.0. Multiplicity issues were addressed for the co-primary outcomes. We adjusted the level of significance by a factor in between a full Bonferroni adjustment and no adjustment at all, because we expected a degree of correlation between the two outcomes; thus, we considered a two-sided P value of $0.05/1.5 = 0.033$ to indicate statistical significance. For the remaining outcome measures, we considered a two-sided P value of less than 0.05 to indicate statistical significance.

Results

Patients

Between September 2014 and August 2015 we assessed 203 patients who fulfilled the inclusion criteria and randomised 153 (75 %) of those (Fig. 1; Fig. 4 in ESM 1); 76 patients were allocated to the fluid restriction group and 77 to the standard care group. One patient in each group withdrew consent for the use of data, thus we analysed data from 151 patients (99 %). Patient characteristics and fluid administration are presented in Table 1 and Table S2 in ESM 1; there appeared to be a degree of imbalance between the two groups for some characteristics, including the rates of pulmonary focus of sepsis and AKI and patient weight.

Fluid protocol

Fifty-five of 75 patients (73 %) in the fluid restriction group vs. 70 of 76 patients (92 %) in the standard care group ($P = 0.002$) received resuscitation fluid during 286 vs. 464 episodes ($P = 0.003$) after randomisation. In the fluid restriction group, the resuscitation fluids were

Table 1 Baseline characteristics

	Fluid restriction group ($n = 75$)	Standard care group ($n = 76$)
Male gender, no. (%)	52 (69)	47 (62)
Age, years	69 (61–76)	73 (67–77)
Weight, kg	80 (65–86)	72 (62–84)
Hypertension, no. (%)	35 (47)	29 (38)
Previous admission for, no. (%)		
Heart failure	11 (15)	15 (20)
Myocardial infarction	10 (13)	7 (9)
Pre-admission plasma creatinine, μmol/L[a]	80 (65–94)	86 (66–100)
Haematological malignancy, no. (%)	5 (7)	8 (11)
Surgery, no. (%)[b]	47 (63)	40 (53)
Source of ICU admittance, no. (%)		
Emergency department	18 (24)	17 (22)
General ward	28 (37)	27 (36)
Operating room or recovery room	27 (36)	29 (38)
Other ICU	2 (3)	3 (4)
Source of sepsis, no. (%)[c]		
Lungs	23 (31)	36 (47)
Abdomen	38 (51)	33 (43)
Urinary tract	8 (11)	10 (13)
Soft tissue	13 (17)	5 (7)
Other	7 (9)	7 (9)
Days from hospital admission to randomisation	1 (0–6)	1 (0–4)
Hours from ICU admission to randomisation	4.5 (2.0–8.5)	4.0 (1.5–6.5)
SAPS II[d]	52 (43–60)	56 (47–66)
SOFA score[e]	10 (7–11)	10 (8–11)
Acute kidney injury, no. (%)[f]	38 (51)	28 (38)
Mechanical ventilation, no. (%)[g]	41 (55)	43 (57)
Highest lactate, mmol/L[h]	3.0 (1.7–4.4)	2.5 (1.5–4.6)
Highest dose of norepinephrine, μg/kg/min[h]	0.25 (0.12–0.40)	0.20 (0.10–0.30)
Highest heart rate, beats/min[h]	106 (95–123)	108 (87–124)
Highest plasma creatinine, μmol/L[i]	133 (84–181)	110 (81–192)
Highest plasma sodium, mmol/L[i]	138 (135–141)	138 (135–141)
Highest plasma potassium, mmol/L[i]	4.2 (3.8–4.9)	4.2 (3.8–4.7)
Fluids given prior to randomisation, mL[i]	4200 (3461–6700)	4790 (3232–6847)

Values with ranges are medians (interquartile ranges)

SI conversion factors: to convert plasma creatinine from μmol/L to mg/dL divide by 88.4; to convert plasma lactate from mmol/L to mg/dL divide by 0.111; to convert plasma sodium and plasma potassium from mmol/L to mEq/L multiply by 1.0

ICU intensive care unit, *SAPS* Simplified Acute Physiology Score, *SOFA* Sequential Organ Failure Assessment

[a] Pre-admission plasma creatinine values were not known in seven patients in the fluid restriction group and six patients in the standard care group; their pre-admission plasma creatinine was estimated from the Modification of Diet in Renal Disease formula using a GFR of 75 mL/min/1.73 m^2

[b] During the hospital admission but prior to randomisation

[c] Some patients had more than one source of infection

[d] SAPS II in the 24 h prior to randomisation. One or 2 of the 17 variables used to calculate the score were missing in 17 patients in the fluid restriction group and 22 patients in the standard care group; their scores were not included here

[e] SOFA score in the 24 h prior to randomisation. One or 2 of the 5 subscores used to calculate the score were missing in 13 patients in the fluid restriction group and 19 patients in the standard care group; their scores were not included here

[f] Acute kidney injury defined as the KDIGO creatinine score >0 in the 24 h prior to randomisation. Data were missing for 1 patient in the fluid restriction group and 2 patients in the standard care group; their scores were not included here

[g] Invasive or non-invasive ventilation in the 24 h prior to randomisation

[h] In the 3 h prior to randomisation

[i] In the 24 h prior to randomisation

administered mainly on the first day after randomisation; in the standard care group, the majority of patients received resuscitation fluid until day 4 (Tables S3, S4 in ESM 1). Resuscitation fluid was given as Ringer's solutions rather than saline in the majority of patients in both groups (Table S4 in ESM 1).

No patients had the fluid resuscitation protocol temporarily suspended (Table S5 in ESM 1), but two patients in each group had the protocol discontinued on the request of surrogates (Fig. 1). Additional details regarding fluid indications, types and timing, co-interventions, haemodynamic variables, and urinary outputs are provided in Tables S3–S11 and Figs. 5–7 in ESM 1.

Primary outcome measures

Cumulated resuscitation fluid volumes given in the ICU at day 5 after randomisation and during the entire ICU stay (the co-primary outcomes) were lower in the fluid restriction group vs. the standard care group [mean differences −1.2 L (95 % CI −2.0 to −0.4); $P < 0.001$ and −1.4 L (95 % CI −2.4 to −0.4); $P < 0.001$, respectively (Table 2; Fig. 2)]. We obtained similar results in the analyses adjusted for the predefined risk factors at baseline, in the per-protocol population (Tables S12, S13 in ESM 1), and in the subgroup analysis of patients who had received more than 5 L of fluid in the 24 h prior to randomisation ($P = 0.91$ for interaction between subgroup and intervention).

Secondary outcome measures

Total fluid inputs and balances in the ICU did not differ with statistical significance between groups either at day 5 after randomisation or during the entire ICU stay (Table 2; Figs. 5, 6 in ESM 1). In the fluid restriction group, 27 of the 75 patients (36 %, 95 % CI 25–47) had a total of 80 violations of the fluid resuscitation protocol (Fig. 8 in ESM 1). The rates of serious adverse reactions to fluids or norepinephrine did not differ between the two intervention groups (Table 2; Table S14 in ESM 1).

Exploratory outcome measures

Death at day 90 (Fig. 3), time to death at latest follow-up (Fig. 3), number of patients with ischaemic events in ICU (Fig. 3; Table S15 in ESM 1), days alive without mechanical ventilation (mean 79 vs. 72 %, $P = 0.48$) or renal replacement therapy (92 vs. 92 %, $P = 0.70$) in the 90-day follow-up period or maximum changes in plasma creatinine in the ICU (median 9 (IQR −13 to 47) vs. 15 (−4 to 62) μmol/L, $P = 0.36$) did not differ with statistical significance between the fluid restriction group and the standard care group. The number of patients with worsening of acute kidney injury in the 90-day period was lower in the fluid restriction group than in the standard care group (Fig. 3; Fig. 10 in ESM 1).

Discussion

We observed that a protocol aimed at restricting resuscitation fluid vs. a protocol aimed at standard care after initial resuscitation of ICU patients with septic shock resulted in lower volumes of resuscitation fluid in the first 5 days and during the entire ICU stay in this binational, multicentre randomised trial. This difference in volumes of resuscitation fluid did not affect fluid balances or rates of serious adverse reactions, use of mechanical ventilation or renal replacement therapy, ischaemia, or death with statistical significance. The number of patients with worsening acute kidney injury appeared to be lower in the fluid restriction group as compared to the standard care group. However, our trial was not powered to show differences in any of these outcomes.

Fluid resuscitation is complex in patients with septic shock and may be influenced by setting, timing, use of haemodynamic triggers and targets and co-interventions as well as focus of infection and co-morbidities [11–13]. The current guideline is based on low level of evidence and recommends a minimum of 30 mL/kg followed by continued fluid resuscitation as long as haemodynamic variables improve [1]. Our fluid restriction protocol challenged in particular the latter part of the guideline. We enrolled ICU patients who had received the 30 mL/kg and observed a median 4.5 L of fluid given prior to randomisation, volumes that are similar to the total fluid volumes given at the end of the 6-h intervention period in the recent early goal-directed therapy trials [14–17]. The patients in those trials were enrolled in emergency departments before any transfer to ICU. In the ICU setting after the initial resuscitation, our fluid restriction protocol resulted in marked reduction in volumes of resuscitation fluid as compared with our standard care protocol where use of resuscitation fluids was continued for some days after randomisation.

We observed lower numbers of patients with worsening acute kidney injury in the fluid restriction group as compared with the standard care group in the exploratory analyses. This may seem counterintuitive; fluids are, in fact, often given by ICU clinicians for oliguria [18]. Our trial was relatively small, and chance or baseline imbalance, including the rates of pulmonary focus of sepsis and AKI at baseline, may have contributed to our results. On the other hand, the results of the recent PROCESS (Protocol-based Care for Early Septic Shock) trial indicated higher volumes of resuscitation fluid given and higher rates of new-onset acute kidney injury in the protocol-based standard therapy group as compared with the early goal-directed therapy group and the usual care

Table 2 Primary and secondary outcome measures

Outcome	Fluid restriction group ($n = 75$)	Standard care group ($n = 76$)	Fluid restriction vs. standard care (95 % CI)[a]	*P* value
Co-primary outcome measures				
Volumes of resuscitation fluid (mL)				
First 5 days after randomisation	500 (0 to 2500) [1687]	2000 (1000 to 4100) [2928]	−1241 (−2043 to −439)	<0.001[b]
During ICU stay after randomisation	500 (0 to 3250) [1992]	2200 (1000 to 4750) [3399]	−1407 (−2358 to −456)	<0.001[b]
Secondary outcome measures				
Total fluid input (mL)[c]				
First 5 days after randomisation	12,411 (5518 to 17,035) [11,777]	13,687 (7163 to 17,082) [12,597]	−820 (−2968 to 1329)	0.45
During ICU stay after randomisation	18,291 (5518 to 34,045) [21,459]	16,970 (7163 to 29,889) [23,495]	−2036 (−10,920 to 6848)	0.65
Cumulated fluid balance (mL)				
First 5 days after randomisation	1752 (−1153 to 3758) [2141]	2680 (407 to 5114) [3289]	−1148 (−2531 to 235)	0.06[b]
During ICU stay after randomisation	1923 (−1964 to 5415) [2,032]	2014 (−168 to 4678) [2507]	−475 (−2254 to 1304)	0.60
Serious adverse reactions[d]				
Number of reactions per day during the ICU stay	0.14 (0 to 0.50) [0.37][e]	0.15 (0 to 0.52) [0.33][e]	NA	0.85[b]

Values in the two intervention groups are presented as medians (interquartile ranges) [estimated mean values adjusted for trial site] unless otherwise specified

A total of 33 patients (8 had died and 25 had been discharged) and 32 (7 had died and 25 had been discharged) were not in the ICU on day 5 in the fluid restriction group and the standard care group, respectively. The ICU length of stay was median 6 days (IQR 3–11) and 5 (3–10) in the fluid restriction group and the standard care group, respectively

CI confidence interval, *NA* not applicable

[a] Estimated mean of the restrictive group minus estimated mean of the standard care group

[b] Non-parametric *p* values. The estimated differences are presented where applicable even though the assumptions for parametric testing were not fully met

[c] The total input of non-resuscitation fluids is presented in Table S16 in ESM 1

[d] Serious adverse reactions to isotonic crystalloids and norepinephrine were recorded daily as anaphylaxis, hypernatraemia, hyperchloraemic acidosis, seizures, central pontine myelinolysis, cerebral haemorrhage, cardiac arrhythmia or delirium

[e] Observed mean presented

group [14]. In two recent cohort studies adherence to the SSC resuscitation bundle, which included administration of a minimum of 30 mL/kg of crystalloids early in septic shock, was associated with improved survival [19, 20]. The interpretation of these data may not conflict that of our data, because our patients had received 30 mL/kg of fluid at enrolment. The results of other cohort studies have associated higher fluid balances in the first days of ICU stay with worse outcomes in patients with sepsis, including those with acute kidney injury [2, 21, 22]. We did not observe statistically significant differences in total fluid input or balances at day 5 most likely because of the large variations in these volumes. Of note, the pretrial power estimation for the detectable difference in total fluid input was more than twofold higher than that of resuscitation fluid. Because we only intervened in administration of resuscitation fluids, statistically significant differences in total fluid inputs were not expected. Also, the observed point estimates for total inputs were similar to those for the volumes of resuscitation fluids. In any case, it is difficult to compare the potential benefits and harms of fluids given for resuscitation and mainly given early with fluids as maintenance and with nutrition and medications during the entire ICU stay. Taken together there is evidence to suggest that lower volumes of resuscitation fluid improve outcomes as compared to higher fluid volumes in patients with septic shock, and to our knowledge there are no high-quality data supporting use of higher fluid volumes in these patients. Given these uncertainties and the abundant use of fluid in patients with septic shock, additional high-quality trials are needed to assess the effects on patient-centred outcomes of protocols aimed at restricting volumes of resuscitation fluid in these patients. Our results indicate that it is feasible to protocolize and restrict resuscitation fluids across multiple ICUs.

Our trial has limitations. The trial was designed to show differences in volumes of resuscitation fluid and was, therefore, relatively small; we could not mask the intervention for investigators, clinicians and patients; and there may have been some baseline imbalance and differences in co-interventions between the two groups. All these factors may have affected the results. Administration of at least 30 mL/kg of fluid had to be documented

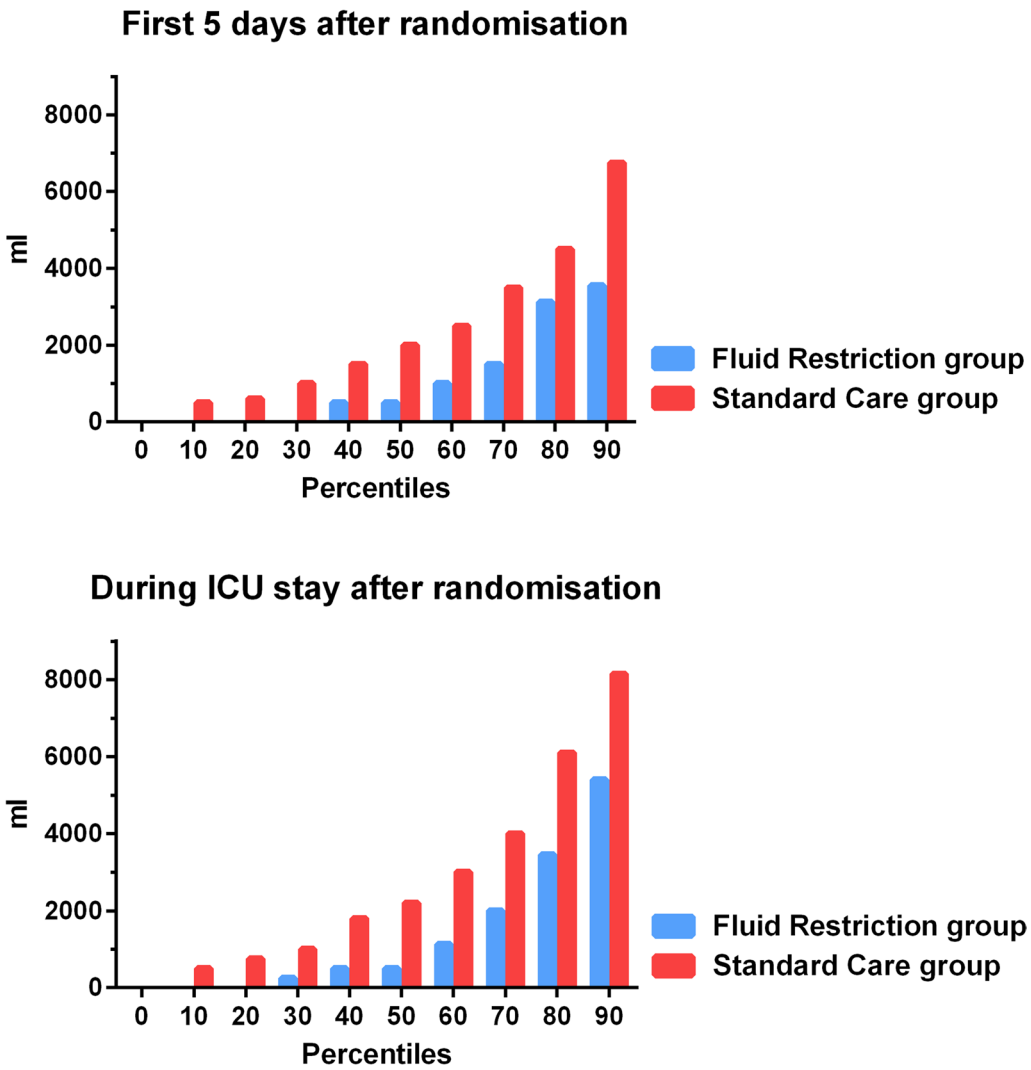

Fig. 2 Percentiles of resuscitations fluids (the primary outcome) given in the restriction group and standard care group after randomisation. Resuscitation fluid was defined as the cumulated volumes of 0.9 % saline, Ringer's lactate, Ringer's acetate and colloid solutions given for circulatory impairment as noted by the clinicians. Lower volumes of resuscitation fluid were given after randomisation in the first 5 days in ICU ($p < 0.001$) and during the entire ICU stay ($p < 0.001$) in the fluid restriction group vs. the standard care group. More detailed analyses are presented in Table 2

prior to inclusion, which may have resulted in selection of specific patient groups. Thus, we may have included more surgical patients than was done in other recent ICU trials in septic shock [23–25]. Use of colloids for circulatory impairment was not allowed in both groups, which might have reduced the external validity of our results. Additionally, we observed a relatively high number of protocol violations, including the administration of resuscitation fluid to patients who did not fulfil the criteria in the fluid restriction group, which reduces the internal and external validity of our results. Also, albumin was administered for circulatory impairment in both groups. In general, protocol violations may be difficult to avoid in trials of complex interventions in ICU [9, 23, 25], and despite these protocol violations we observed separation in resuscitation fluid volumes between the two intervention groups. Potential measures to lessen the number protocol violations in a large-scale trial include promoting the results of the present trial, which did not indicate safety concerns, and allowing resuscitation fluid in the restriction group on the basis of tachycardia and a lower lactate threshold.

The strengths of our trial include lower risk of bias as group allocation was concealed and the statistician adhered to the predefined statistical analysis plan while blinded to the intervention. It is reasonable to assume

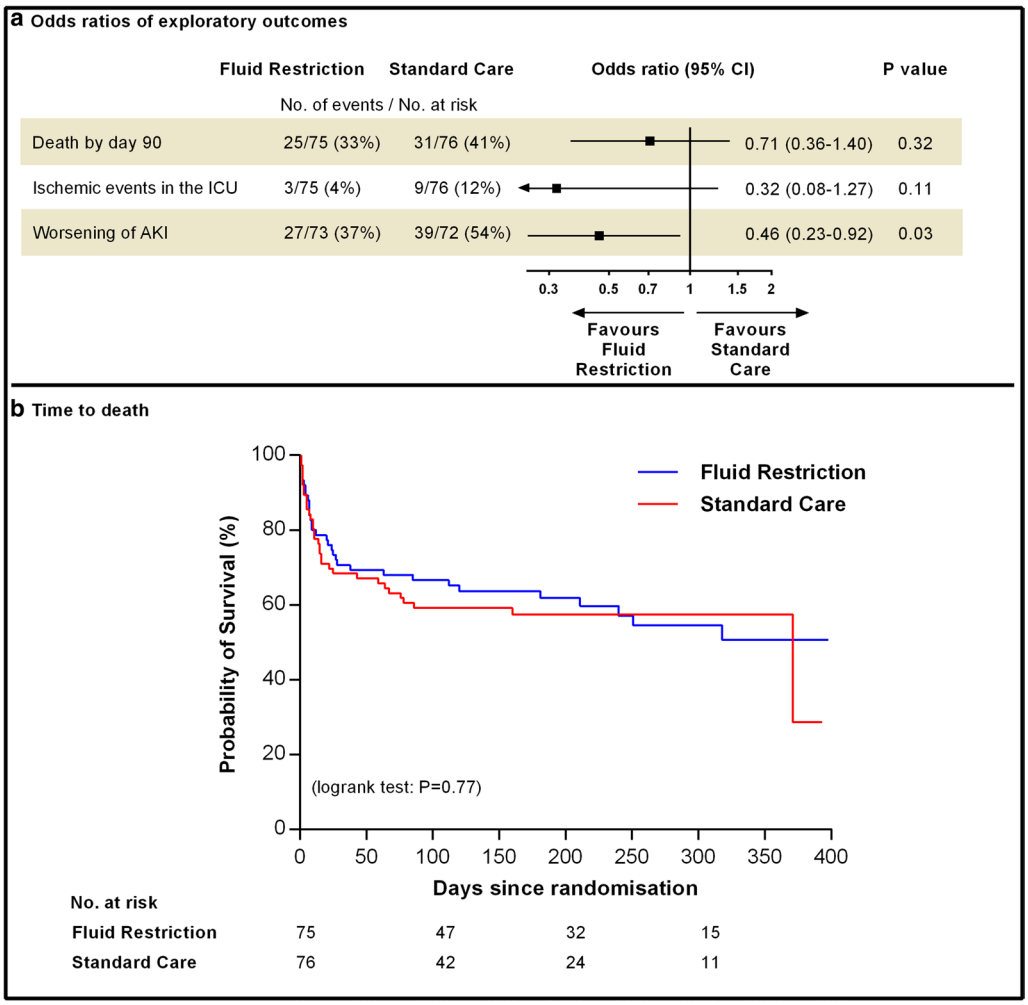

Fig. 3 Exploratory outcome measures. **a** Odds ratios (*black boxes*) with 95 % confidence intervals (*horizontal lines*) for the binary explorative outcomes in the fluid restriction group vs. the standard care group as assessed by logistic regression analyses with adjustment for the stratification variable (trial site). Ischaemic events were defined as at least one of the following during ICU stay: intestinal, limb, ischaemia or myocardial ischaemia. Worsening acute kidney injury (AKI) was defined as worsening of the KDIGO stage (plasma creatinine criteria or use of renal replacement therapy). A total of 6 patients (4 %) had either missing baseline plasma creatinine or did not have any plasma creatinine measurements during ICU stay—these patients were not included in the above complete case analysis. Since *p* of Little's test was less than 0.001 and one auxiliary variable (rate of serious adverse reactions) was highly correlated ($|r| = 0.53$) with worsening of KDIGO we did multiple (monotone) imputation. The results were comparable to that of the complete case analysis including that of the inference ($p = 0.03$). On the request of reviewers, we conducted a post hoc sensitivity analysis excluding patients with KDIGO stage 3 at baseline from the analysis of worsening of AKI; excluding these patients, 27/66 vs. 39/68 had worsening of AKI in the fluid restriction group vs. standard care group [odds ratio 0.52 (95 % confidence interval (CI) 0.26–1.02; $p = 0.058$)]. **b** Survival curves and the number of patients at risk censored at the time of follow-up of the last randomised patient (4 November 2015) for the two intervention groups. The median time of follow-up was 262 days (interquartile range 173–326). *P* of the logrank test was 0.77. Using the Cox analysis adjusted by the stratification variable (site) the hazard ratio between the fluid restriction group and the standard care group was 0.89 (CI 0.54–1.45; $p = 0.64$)

that our results are generalizable, because patients were recruited in both university and non-university hospitals and the majority of patients screened were included. In addition, most patient characteristics and outcome rates were comparable to those of some recent trials in ICU patients with septic shock [23–25].

In conclusion, a protocol aimed at restricting resuscitation fluid was feasible and resulted in reduced volumes of resuscitation fluid as compared with a protocol aimed at standard care in ICU patients with septic shock who had undergone initial resuscitation. As the exploratory outcomes suggested benefit from fluid restriction

and fluid is abundantly used in septic shock, we need large, high-quality trials assessing benefits vs. harms of lower vs. higher volumes of resuscitation fluid in these patients.

Electronic supplementary material
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Compliance with ethical standards

Conflicts of interest
The Department of Intensive Care, Rigshospitalet receives research funds from CSL Behring, Switzerland, Fresenius Kabi, Germany, and Ferring Pharmaceuticals, Denmark.

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References

1. Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, Sevransky JE, Sprung CL, Douglas IS, Jaeschke R, Osborn TM, Nunnally ME, Townsend SR, Reinhart K, Kleinpell RM, Angus DC, Deutschman CS, Machado FR, Rubenfeld GD, Webb S, Beale RJ, Vincent JL, Moreno R (2013) Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012. Intensive Care Med 39:165–228
2. Boyd JH, Forbes J, Nakada TA, Walley KR, Russell JA (2011) Fluid resuscitation in septic shock: a positive fluid balance and elevated central venous pressure are associated with increased mortality. Crit Care Med 39:259–265
3. Acheampong A, Vincent JL (2015) A positive fluid balance is an independent prognostic factor in patients with sepsis. Crit Care 19:251
4. Maitland K, Kiguli S, Opoka RO, Engoru C, Olupot-Olupot P, Akech SO, Nyeko R, Mtove G, Reyburn H, Lang T, Brent B, Evans JA, Tibenderana JK, Crawley J, Russell EC, Levin M, Babiker AG, Gibb DM (2011) Mortality after fluid bolus in African children with severe infection. N Engl J Med 364:2483–2495
5. Hjortrup PB, Haase N, Wetterslev J, Perner A (2016) Associations of hospital and patient characteristics with fluid resuscitation volumes in patients with severe sepsis: post hoc analyses of data from a multicentre randomised clinical trial. PLoS One 11:e0155767
6. Ait-Oufella H, Lemoinne S, Boelle PY, Galbois A, Baudel JL, Lemant J, Joffre J, Margetis D, Guidet B, Maury E, Offenstadt G (2011) Mottling score predicts survival in septic shock. Intensive Care Med 37:801–807
7. Wacharasint P, Nakada TA, Boyd JH, Russell JA, Walley KR (2012) Normal-range blood lactate concentration in septic shock is prognostic and predictive. Shock 38:4–10
8. Kellum JA, Lameire N (2013) Diagnosis, evaluation, and management of acute kidney injury: a KDIGO summary (Part 1). Crit Care 17:204
9. Perner A, Haase N, Guttormsen AB, Tenhunen J, Klemenzson G, Aneman A, Madsen KR, Moller MH, Elkjaer JM, Poulsen LM, Bendtsen A, Winding R, Steensen M, Berezowicz P, Soe-Jensen P, Bestle M, Strand K, Wiis J, White JO, Thornberg KJ, Quist L, Nielsen J, Andersen LH, Holst LB, Thormar K, Kjaeldgaard AL, Fabritius ML, Mondrup F, Pott FC, Moller TP, Winkel P, Wetterslev J (2012) Hydroxyethyl starch 130/0.42 versus Ringer's acetate in severe sepsis. N Engl J Med 367:124–134
10. ICH Steering Committee (1998) International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use. ICH Harmonised Tripartite Guideline for Statistical Principles for Clinical Trials, 3rd edn. Brookwood Medical Publications, London
11. Seymour CW, Rosengart MR (2015) Septic shock: advances in diagnosis and treatment. JAMA 314:708–717
12. Perner A, Vieillard-Baron A, Bakker J (2015) Fluid resuscitation in ICU patients: quo vadis? Intensive Care Med 41:1667–1669
13. Myburgh JA, Mythen MG (2013) Resuscitation fluids. N Engl J Med 369:1243–1251
14. Yealy DM, Kellum JA, Huang DT, Barnato AE, Weissfeld LA, Pike F, Terndrup T, Wang HE, Hou PC, LoVecchio F, Filbin MR, Shapiro NI, Angus DC (2014) A randomized trial of protocol-based care for early septic shock. N Engl J Med 370:1683–1693
15. Peake SL, Delaney A, Bailey M, Bellomo R, Cameron PA, Cooper DJ, Higgins AM, Holdgate A, Howe BD, Webb SA, Williams P (2014) Goal-directed resuscitation for patients with early septic shock. N Engl J Med 371:1496–1506
16. Mouncey PR, Osborn TM, Power GS, Harrison DA, Sadique MZ, Grieve RD, Jahan R, Harvey SE, Bell D, Bion JF, Coats TJ, Singer M, Young JD, Rowan KM (2015) Trial of early, goal-directed resuscitation for septic shock. N Engl J Med 372:1301–1311
17. Angus DC, Barnato AE, Bell D, Bellomo R, Chong CR, Coats TJ, Davies A, Delaney A, Harrison DA, Holdgate A, Howe B, Huang DT, Iwashyna T, Kellum JA, Peake SL, Pike F, Reade MC, Rowan KM, Singer M, Webb SA, Weissfeld LA, Yealy DM, Young JD (2015) A systematic review and meta-analysis of early goal-directed therapy for septic shock: the ARISE, ProCESS and ProMISe Investigators. Intensive Care Med 41:1549–1560
18. Cecconi M, Hofer C, Teboul JL, Pettila V, Wilkman E, Molnar Z, Della RG, Aldecoa C, Artigas A, Jog S, Sander M, Spies C, Lefrant JY, De BD (2015) Fluid challenges in intensive care: the FENICE study: a global inception cohort study. Intensive Care Med 41:1529–1537
19. Levy MM, Rhodes A, Phillips GS, Townsend SR, Schorr CA, Beale R, Osborn T, Lemeshow S, Chiche JD, Artigas A, Dellinger RP (2014) Surviving Sepsis Campaign: association between performance metrics and outcomes in a 7.5-year study. Intensive Care Med 40:1623–1633

20. Rhodes A, Phillips G, Beale R, Cecconi M, Chiche JD, De Backer D, Divatia J, Du B, Evans L, Ferrer R, Girardis M, Koulenti D, Machado F, Simpson SQ, Tan CC, Wittebole X, Levy M (2015) The Surviving Sepsis Campaign bundles and outcome: results from the International Multicentre Prevalence Study on Sepsis (the IMPreSS study). Intensive Care Med 41:1620–1628
21. Vincent JL, Sakr Y, Sprung CL, Ranieri VM, Reinhart K, Gerlach H, Moreno R, Carlet J, Jr Le Gall, Payen D (2006) Sepsis in European intensive care units: results of the SOAP study. Crit Care Med 34:344–353
22. Vaara ST, Korhonen AM, Kaukonen KM, Nisula S, Inkinen O, Hoppu S, Laurila JJ, Mildh L, Reinikainen M, Lund V, Parviainen I, Pettila V (2012) Fluid overload is associated with an increased risk for 90-day mortality in critically ill patients with renal replacement therapy: data from the prospective FINNAKI study. Crit Care 16:R197
23. Holst LB, Haase N, Wetterslev J, Wernerman J, Guttormsen AB, Karlsson S, Johansson PI, Aneman A, Vang ML, Winding R, Nebrich L, Nibro HL, Rasmussen BS, Lauridsen JR, Nielsen JS, Oldner A, Pettila V, Cronhjort MB, Andersen LH, Pedersen UG, Reiter N, Wiis J, White JO, Russell L, Thornberg KJ, Hjortrup PB, Muller RG, Moller MH, Steensen M, Tjader I, Kilsand K, Odeberg-Wernerman S, Sjobo B, Bundgaard H, Thyo MA, Lodahl D, Maerkedahl R, Albeck C, Illum D, Kruse M, Winkel P, Perner A (2014) Lower versus higher hemoglobin threshold for transfusion in septic shock. N Engl J Med 371:1381–1391
24. Caironi P, Tognoni G, Masson S, Fumagalli R, Pesenti A, Romero M, Fanizza C, Caspani L, Faenza S, Grasselli G, Iapichino G, Antonelli M, Parrini V, Fiore G, Latini R, Gattinoni L (2014) Albumin replacement in patients with severe sepsis or septic shock. N Engl J Med 370:1412–1421
25. Asfar P, Meziani F, Hamel JF, Grelon F, Megarbane B, Anguel N, Mira JP, Dequin PF, Gergaud S, Weiss N, Legay F, Le TY, Conrad M, Robert R, Gonzalez F, Guitton C, Tamion F, Tonnelier JM, Guezennec P, Van Der Linden T, Vieillard-Baron A, Mariotte E, Pradel G, Lesieur O, Ricard JD, Herve F, du CD, Guerin C, Mercat A, Teboul JL, Radermacher P (2014) High versus low blood-pressure target in patients with septic shock. N Engl J Med 370:1583–1593

CLASSIC Trial Supplement 1

Table of Contents

Methods

Classic Trial definition of resuscitation fluid

Resuscitation fluid was defined as 0.9% saline, Ringer's lactate, Ringer's acetate or colloid solutions given in the ICU for circulatory impairment as noted by clinicians on a bedside trial specific chart.

Classic Trial definition of Acute Kidney Injury (AKI)

AKI was defined according to the KDIGO serum creatinine criteria.[1] Urine output criteria were not assessed.

KDIGO Staging	**Serum creatinine criteria**
Stage	
Stage 1	1.5–1.9 times baseline (pre-admission creatinine) OR ≥0.3 mg/dl (≥26.5 µmol/l) increase
Stage 2	2.0–2.9 times baseline (pre-admission creatinine)
Stage 3	3.0 times baseline (pre-admission creatinine) OR Increase in serum creatinine to ≥4.0 mg/dl (≥353.6µmol/l) OR Initiation of renal replacement therapy

Handling of missing pre-admission creatinine

During the monitoring of missing values it was noted that 13 (9%) of the habitual p-creatinine (pre-admission creatinine) values were missing in the Classic Trial – 7 (9%) in the Fluid Restriction group and 6 (8%) in the Standard Care group. In these cases the MDRD equation was used to estimate the pre-admission creatinine from a eGFR of 75 ml/min per 1.73 m^2 as recommended in the KDIGO guidelines[1].

Classic Trial definitions of serious adverse reactions (SAR).

Investigators were asked daily to register events of serious adverse reactions. A suspected or confirmed association between the event and the drug was not required.

Serious adverse reactions to isotonic crystalloids (0.9% Saline or Ringer's solutions):

Hypernatriemia (defined as plasma Natrium (sodium) above 155 mmol/l).

Hyperchloremic acidosis (defined as pH below 7.35 AND plasma chloride above 110 mmol/l).

Seizures (clinical signs OR verified by electroencephalogram).

Central pontine myelinolysis verified by CT or MR scan.

Anaphylactic reaction (defined as urticaria and at least one of the following: Worsened circulation (>20% decrease in blood pressure or >20% increase in vasopressor dose), increased airway resistance (>20 % increase in peak pressure on the ventilator), clinical stridor or bronchospasm, subsequent treatment with bronchodilators).

Serious adverse reactions to norepinephrine:

Cardiac arrhythmia (supraventricular or ventricular tachyarrhythmia) resulting in intervention (drugs or electrical cardioversion) against it.

Psychiatric symptoms (delirium, hallucinations, delusions) resulting in medication.

Intracerebral hemorrhage verified by CT or MR scan.

Classic Trial definitions of ischemic events.

Limb ischemia: clinical signs and at least one of the following: initiation/increased antithrombotic treatment, open/percutaneous intervention or amputation

Intestinal ischemia: verified by endoscopy or open surgery.

Myocardial ischemia: diagnosis of acute myocardial infarction (ST-elevation myocardial infarction or non-ST-elevation myocardial infarction) or unstable angina pectoris, according to the criteria in the clinical setting in question (elevated biomarkers, ischemic signs on electrocardiogram and/or clinical presentation).

Cerebral ischemia: verified by CT or MR scan.

Classic Trial inclusion criteria

- Adult intensive care patients (age ≥ 18 years)

AND

- Sepsis defined as at least 2 of 4 SIRS criteria fulfilled within 24 hours according to Society of Critical Care Medicine/American College of Chest Physicians (SCCM/ACCP)[2]:

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. Use the most deranged value recorded* ***in the 24 hours before randomization****.*
2. ***HEART RATE ≥90 beats/minute****. If patient has 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 randomization****.*
3. ***MECHANICAL VENTILATION*** *for an acute process or respiratory rate ≥ 20 breaths per minute or a $PaCO_2$ < 4.3 kPa (32 mmHg). Use the most deranged respiratory rate or $PaCO_2$ recorded* ***in the 24 hours before randomization****.*
4. ***WHITE BLOOD CELL COUNT of ≥12 x 10^9/l or ≤ 4 x 10^9/l****. Use the most deranged value recorded* ***in the 24 hours before randomization****.*

AND

- Suspected or confirmed site of infection OR positive blood culture

AND

- Suspected or confirmed circulatory impairment (hypotension/hypoperfusion/hypovolemia) for no more than 12 hours including the hours preceding ICU admission. Circulatory impairment defined as at least one of the following: Systolic blood pressure < 90 mmHg, heart rate > 140 beats/min, lactate ≥ 4 mmol/l, OR use of vasopressors.

AND

- At least 30 ml/kg ideal bodyweight fluid (colloids, crystalloids or blood products) given in the last 6 hours

AND

- Shock defined as ongoing infusion of noradrenaline (any dose) to maintain blood pressure

Classic Trial exclusion criteria

1. Use of any form of renal replacement therapy (RRT) or RRT deemed imminent by ICU physician, i.e. RRT would start within 6 hours
2. Severe hyperkalemia (p-K > 6 mmol/l) within the last 6 hours.
3. Plasma creatinine > 350 µmol/l.
4. Invasively ventilated with FiO_2 > 0.80 and positive end-expiratory pressure (PEEP) > 10 cmH_2O
5. Life-threatening bleeding defined as 1) the presence of haemorrhagic shock, as judged by researcher or treating clinicians or 2) the need for surgical procedure, incl. endoscopy to maintain haemoglobin level.
6. Kidney or liver transplant during present admission.
7. Burns > 10% body surface area
8. Previously enrolled in the CLASSIC trial and had finished the 90 day observation period.
9. Patients for whom it had been decided not to give full life support including mechanical ventilation and RRT
10. Consent not obtainable.

Suggestions for use of co-interventions in Classic

Treatment of septic shock is complex with multiple interventions. To minimize potential differences in the use of co-interventions suggestions are given for use of the following interventions based on the Surviving Sepsis Campaigns (SSC) 2012 guidelines:[3]

- **Vasopressors**: Noradrenaline as first choice vasopressor. Adrenaline may be added if additional medication is needed to achieve adequate blood pressure.
- **Inotropes**: Dobutamine may be given or added to vasopressors if 1) myocardial dysfunction or b) ongoing signs of hypoperfusion despite adequate intravascular volume and adequate MAP is achieved.
- **Glucocorticoids**: Shock reversal glucocorticoids are suggested only to be administered when hemodynamic stability cannot be achieved - i.e. MAP remains below target despite increasing vasopressor dose.
- **Blood products:**

  Red blood cells (RBCs): A hemoglobin threshold of 4.3 mmol/l (7 g/dl) is suggested in absence of life-threatening bleeding and myocardial infarction.

  Fresh frozen plasma (FFP) should only be administered in presence of bleeding or planned invasive procedures AND signs of coagulopathy (e.g. INR > 2 OR TEG R-time > 10 seconds).

  Platelets: Suggestions for platelet thresholds: In absence of bleeding: $< 10 \times 10^9$/l, significant risk of bleeding: $< 20 \times 10^9$/l and for active bleeding, surgery or invasive procedures: $< 50 \times 10^9$/l.
- **Renal replacement therapy (e.g. dialysis and hemofiltration)**: Suggestions for indications for RRT: Clinically significant volume overload not responding to diuretics, progressive uremia with s-urea > 30 mmol/l, hyperkalemia with s-K > 6.0 mmol/l and rising, hypernatremia with s-Na > 160 mmol/l, metabolic acidosis with pH < 7.1 not controlled by bicarbonate infusion ($NaHCO_3$), plasma-creatinine > 350 µmol/l OR hypercalcemia with s-Ca > 4.00 mmol/l.

  Use of RRT on sepsis indication alone should not be performed in Classic.
- **Interventions against atrial fibrillation/flutter**: Fluid bolus as intervention against atrial fibrillation/flutter is not recommended. Rather cardioversion/frequency modulation with magnesium, digoxin, amiodarone and/or DC cardioversion is recommended.

General fluid management in Classic

The patients in Classic are randomised to one of two approaches to fluid therapy; restrictive use of resuscitation fluid or standard care.
For **all** patients in Classic the following apply:

- **Crystalloids (e.g. Saline (0.9 %), Ringer's solutions and Plasmalyte)**: The isotonic crystalloids should be the only choice for resuscitation purposes. It is further recommended that these solutions to the extent possible only are used for resuscitation purposes (see below).

- **Albumin and synthetic colloids**: Both human albumin and the synthetic colloids should not be administered in Classic. Examples of synthetic colloids: Hydroxyethyl starches (HES): Voluven® and Tetraspan ®, Dextrans: Macrodex®, Gelatine: Gelofusine®.

- **Electrolyte correction**: Electrolyte correction should to the extent possible be handled enterally. If not possible, strive to use other fluids than the isotonic crystalloids.

- **Overt fluid losses (e.g. diarrhoea, bleeding, ascites drainage, pleural tap)** may be replenished. To the extent possible avoid use of the isotonic crystalloids.

- **Water supplements:** Should to the extent possible be handled enterally. If not possible and total fluid input (incl. fluid with medications and nutrition) is below 1500 ml/day, then intravenous isotonic glucose may administered as supplement.

- **Life-threatening bleeding.** In presence of life-threatening bleeding (defined as presence of haemorrhagic shock as judged by investigator or treating clinician) the Classic protocol is temporarily suspended. The Classic protocol is to be resumed as soon as the suspension criterion is no longer fulfilled.

- **Fluids with medication** are handled as usual.

- **Fluids with nutrition** are handled as usual.

Table S1. Patients with protocol violations

	Fluid restriction Group (N=75)	**Standard Care Group (N=76)**
Criteria	No. / total no. (%)	No. / total no. (%)
Inclusion criterion not fulfilled*	5/75 (7%)	3/76 (4%)
Exclusion criterion fulfilled†	1/75 (1%)	0/75 (0%)
Intervention stopped upon request of patient or next of kin	2/75 (3%)	2/76 (3%)
Fluid resuscitation bolus given without fulfilment of Classic criteria ‡	27/75 (36%)	NA
Any colloid resuscitation episode[d]	16/75 (21%)	18/76 (24%)
Total no. of patients with one or more protocol violations	34/75 (45%)	23/76 (30%)

Abbreviations: NA, not applicable

* During GCP-monitoring of the included patients 1 patient in the fluid restriction group and 2 patients in the standard care group were found to have had circulatory impairment for more than 12 hours prior to randomization. Additionally 4 patients in the fluid restriction group and 1 patient in the standard care group was found not to have received at least 30 ml/kg fluid in the 6 hours prior to randomization with sufficient documentation.

† During GCP-monitoring of the included patients 1 patient in the fluid restriction group was found to have burns > 10% (patient records described 11% burn injury of total body surface area).

‡ CLASSIC criteria: Lactate 4 mmol/L or above, MAP below 50 mmHg, mottling beyond edge of kneecap OR severe oliguria (urinary output 0.1 mL/kg/hour or below, but only during the first 2 hours after randomization).

§ Any use of colloid solutions (Albumin or synthetic colloids) for resuscitation after randomization.

Table S2. Additional baseline characteristics

	Fluid Restriction Group (N=75)		Standard Care Group (N=76)	
Variable	No. received/ no. at risk (%)*	Values for all patients at risk - mL	No. received/ no. at risk (%)*	Values for all patients at risk - mL
Fluids given in the 24 hours prior to randomization				
Isotonic crystalloids†	73/75 (97%)	3,800 (2,200-5,600)	74/75 (99%)	4,000 (2,500-5,300)
Synthetic colloids ‡	0/75 (0%)	0 (0-0)	2/75 (3%)	0 (0-0)
Albumin 5% or 20%	29/75 (39%)	0 (0-250)	21/75 (28%)	0 (0-100)
Red blood cells	12/75 (16%)	0 (0-0)	10/75 (13%)	0 (0-0)
Fresh frozen plasma	7/75 (9%)	0 (0-0)	4/75 (5%)	0 (0-0)
Platelets	7/75 (9%)	0 (0-0)	5/75 (7%)	0 (0-0)
Other fluids	58/75 (77%)	500 (55-1,010)	56/74 (76%)	380 (23-900)
Hemodynamic variables - lowest values in the 3 hours prior to randomization				
	No. assessed/ no. at risk (%)	Values for patients assessed only	No. assessed/ no. at risk (%)	Values for patients assessed only
CVP, mmHg	8/75 (11%)	9 (7-15)	12/76 (16%)	9 (6-11)
$ScvO_2$, %	27/75 (36%)	72 (66-78)	24/76 (32%)	74 (66-80)

All values are presented as median (interquartile range).

Abbreviations: CVP, central venous pressure. $ScvO_2$, central venous oxygen saturation.

* No. receiving is those patients who did receive the specific solution. No. at risk is those patients who had registered data. Where the no. at risk is below all patients allocated to the group, this is due to missing data. We had the following missing data: one patient in the standard care group had missing data for all fluids given prior to randomization; one additional patient in the standard care group had missing "other fluids" given prior to randomization. Complete cases are given.

† Isotonic crystalloids: 0.9% saline, Ringer's acetate and Ringer's lactate solutions.

‡ Synthetic colloids: hydroxyethyl starch, dextran and gelatin solutions.

Table S3. Crystalloid and colloid solutions given as resuscitation fluid and non-resuscitation fluid

	Fluid Restriction Group (N=75)		Standard Care Group (N=76)	
Variable	No. received/ no. at risk (%)*	Volume received for all patients at risk - mL	No. received/ no. at risk (%)*	Volume received for all patients at risk - mL
Resuscitation fluid – crystalloid and colloid solutions				
Day 1-5†	51/75 (68%)	500 (0-2,500)	70/76 (92%)	2,000 (1,000-4,100)
Total‡	55 /75 (73%)	500 (0-3,250)	70/76 (92%)	2,200 (1,000-4,750)
Non-resuscitation fluid – crystalloid and colloid solutions§				
Day 1-5†	16/75 (21%)	0 (0-0)	12/76 (16%)	0 (0-0)
Total‡	24/75 (32%)	0 (0-500)	21/76 (28%)	0 (0-500)
Indication not registered – crystalloid and colloid solutions‖				
Day 1-5†	39/75 (52%)	17 (0-676)	46/76 (61%)	407 (0-1,200)
Total‡	42/75 (56%)	150 (0-1,000)	50/76 (66%)	525 (0-1,660)

All volumes are presented as median (interquartile range).

Investigators and clinicians were asked to register on a trial specific bedside form the indication(s) for each administration of isotonic crystalloids (0.9% saline or Ringer's solutions) or colloids (albumin or synthetic colloid solutions).

* No. receiving is those patients who received any fluid in the specific category on the given days. No. at risk is those patients who were allocated to the intervention group.

† Cumulative data for day 1 to day 5.

‡ Cumulative data for the full trial period in the ICU to a maximum of 90 days after randomization

§ Non-resuscitation indications included replacement of overt losses (diarrhea, pleural-/ascites drainage, gastric aspirate etc.), forced diuresis (e.g. for myoglobinemia), non-life threatening bleeding, correction of electrolytes, and too aggressive fluid removal during renal replacement therapy.

‖ Fluid input was registered from source data, but no indications were registered on the trial specific bedside fluid indication form.

Table S4. Crystalloids and colloids by type and fluid balances after randomization

	Fluid Restriction Group (N=75)		Standard Care Group (N=76)	
Variable	No. received/ no. at risk (%)*	Volume received for all patients at risk - mL	No. received/ no. at risk (%)*	Volume received for all patients at risk - mL
Ringer's solutions				
Day 1†	43/75 (57%)	500 (0-1,000)	47/76 (62%)	500 (0-1,205)
Day 2	25/71 (35%)	0 (0-500)	50/74 (68%)	591 (0-2,000)
Day 3	15/62 (24%)	0 (0-0)	32/62 (52%)	175 (0-1,000)
Day 4	10/54 (19%)	0 (0-0)	28/53 (53%)	197 (0-1,000)
Day 5	10/42 (24%)	0 (0-0)	13/44 (30%)	0 (0-278)
Day 1-5‡	58/75 (77%)	1,000 (170-2,250)	68/76 (89%)	2,000 (1,000-4,647)
Total§	62 /75 (83%)	1,200 (481-3,500)	68/76 (89%)	3,000 (1,000-6,775)
0.9% Saline				
Day 1†	15/75 (20%)	0 (0-0)	31/76 (41%)	0 (0-500)
Day 2	8/71 (11%)	0 (0-0)	19/74 (26%)	0(0-100)
Day 3	4/62 (6%)	0 (0-0)	11/62 (18%)	0 (0-0)
Day 4	4/54 (7%)	0 (0-0)	11/53 (21%)	0 (0-0)
Day 5	1/42 (2%)	0 (0-0)	6/44 (14%)	0 (0-0)
Day 1-5‡	21/75 (28%)	0 (0-300)	38/76 (50%)	13 (0-1,123)
Total§	24/75 (32%)	0 (0-400)	42/76 (55%)	225 (0-1,500)
Synthetic colloids - hydroxyethyl starch, dextran and gelatin solutions				
Total§	0/75 (0%)	0 (0-0)	0/76 (0%)	0 (0-0)
Albumin 5% or 20%				
Day 1†	6/75 (8%)	0 (0-0)	9/76 (12%)	0 (0-0)
Day 2	5/71 (7%)	0 (0-0)	13/74 (18%)	0 (0-0)
Day 3	6/62 (10%)	0 (0-0)	7/62 (11%)	0 (0-0)
Day 4	3/54 (6%)	0 (0-0)	3/53 (6%)	0 (0-0)
Day 5	4/42 (10%)	0 (0-0)	5/44 (11%)	0 (0-0)
Day 1-5‡	17/75 (23%)	0 (0-0)	24/76 (32%)	0 (0-100)
Total§	28/75 (37%)	0 (0-250)	31/76 (41%)	0 (0-350)
Fluid balance				
	No. with data/ no. in ICU	Fluid balance - ml	No. with data/ no. in ICU	Fluid balance - ml
Day 1†	75/75	556 (-263-2,187)	76/76	888 (22-2,279)
Day 2	71/71	610 (-460-1,847)	74/74	976 (412-2,313)

Day 3	62/62	165 (-662-1,395)	62/62	214 (-499-1,555)
Day 4	54/54	-60 (-1,185-645)	53/53	0 (-952-1,024)
Day 5	42/42	-60 (-1,057-623)	44/44	59 (-830-717)
Day 1-5‡	75/75	1,752 (-1,153-3,758)	76/76	2,680 (407-5,114)
Total§	75/75	1,923 (-1,964-5,415)	76/76	2,014 (-168-4,678)

All volumes presented as median (interquartile range).

Abbreviations: NA, not applicable

* No. receiving is those patients who did receive the specific solution on the given day(s). No. at risk is those patients who had registered data on that day(s). Where the no. at risk is below all patients allocated to the group, this is due to death or ICU discharge.

† The first day was from the time of randomization to the next start of the specific ICU's 24-hour fluid chart and lasted median 12 (IQR 7-17) hours in the fluid restriction group and 10 (5-16) hours in standard care group.

‡ Cumulative data for day 1 to day 5.

§ Cumulative data for the full trial period in ICU to a maximum of 90 days after randomization

Table S5. Temporary protocol suspension due to life-threatening bleeding

	Fluid Restriction Group (N=75)	**Standard Care Group (N=76)**
	No. with suspension/ no. at risk (%)	No. with suspension/ no. at risk (%)
Suspensions	0/75 (0%)	0/76 (0%)

The protocol could temporarily be suspended in case of life-threatening bleeding as judged by treating physicians.

Table S6. Other types of fluids given after randomization

	Fluid Restriction Group (N=75)		Standard Care Group (N=76)	
Variable	No. received/ no. at risk (%)*	Volume received for all patients at risk - mL	No. received/ no. at risk (%)*	Volume received for all patients at risk - mL
Blood products - red blood cells, fresh frozen plasma and platelets				
Day 1†	11/75 (15%)	0 (0-0)	2/76 (3%)	0 (0-0)
Day 2	8/71 (11%)	0 (0-0)	9/74 (12%)	0 (0-0)
Day 3	7/62 (11%)	0 (0-0)	7/62 (11%)	0 (0-0)
Day 4	6/54 (11%)	0 (0-0)	5/53 (9%)	0 (0-0)
Day 5	7/42 (17%)	0 (0-0)	4/44 (9%)	0 (0-0)
Day 1-5‡	24/75 (32%)	0 (0-300)	19/76 (25%)	0 (0-123)
Total§	30/75 (40%)	0 (0-600)	27/76 (36%)	0 (0-562)
Nutrition - enteral and parenteral				
Day 1†	39/75 (52%)	100 (0-500)	40/75 (53%)	105(0-400)
Day 2	58/71 (82%)	720 (136-1,440)	57/74 (77%)	500 (80-1,325)
Day 3	54/62 (87%)	848 (445-1,498)	57/62 (92%)	931 (400-1,460)
Day 4	49/54 (91%)	1,004 (490-1,438)	49/53 (92%)	960 (596-1,485)
Day 5	41/42 (98%)	1,091 (663-1,512)	41/44 (93%)	1,013 (497-1,313)
Day 1-5‡	69/75 (92%)	2,790 (1,031-5,350)	70/75 (93%)	2,740 (820-4,617)
Total§	69/75 (92%)	5,010 (1,617-11,305)	70/75 (93%)	4,858 (1,312-8,983)
Fluids with medication				
Day 1†	72/75 (96%)	649 (347-1,072)	70/75 (93%)	541 (200-1,012)
Day 2	67/71 (94%)	1,349 (855-2,029)	73/74 (99%)	1,182 (675-1,770)
Day 3	59/62 (95%)	1,259 (685-1,973)	59/62 (95%)	1,077 (700-1,568)
Day 4	51/54 (94%)	1,197 (617-1,706)	52/53 (98%)	1,006 (690-1,340)
Day 5	42/42 (100%)	1,315 (635-1,847)	42/44 (95%)	935 (520-1,385)
Day 1-5‡	75/75 (100%)	4,618 (1,717-7,580)	75/75 (100%)	3,883 (1,362-6,050)
Total§	75/75 (100%)	5,302 (1,717-12,998)	75/75 (100%)	5,248 (1,665-9,243)
Other fluids‖				
Day 1†	30/75 (40%)	0 (0-360)	33/75 (44%)	0 (0-250)
Day 2	38/71 (54%)	40 (0-500)	38/74 (51%)	20 (0-450)
Day 3	24/62 (39%)	0 (0-450)	30/62 (48%)	0 (0-245)
Day 4	22/54 (41%)	0 (0-250)	26/53 (49%)	0 (0-300)
Day 5	19/42 (45%)	0 (0-280)	23/44 (52%)	30 (0-400)
Day 1-5‡	49/75 (65%)	400 (0-1,980)	60/75 (80%)	660 (80-1,650)

Total§	58/75 (77%)	1,180 (88-3,809)	66/75 (88%)	1,400 (220-3,145)

All volumes presented as median (interquartile range).

* No. receiving is those patients who did receive the specific solution on the given day(s). No. at risk is those patients who had registered data on that day(s). Where the no. at risk is below all patients allocated to the group, this is due to death, ICU discharge or missing data. We had the following missing data: data on nutrition, fluids with medication and "other fluids" were missing for one patient on one day (day 1) in the standard care group. Complete cases are given.

† The first day was from the time of randomization to the next start of the specific ICU's 24-hour fluid chart and lasted median 12 (IQR, 7-17) hours in the fluid restriction group and 10 (5-16) hours in standard care group.

‡ Cumulative data for day 1 to day 5.

§ Cumulative data for the full trial period in ICU to a maximum of 90 days after randomization.

|| Combined water, <10% glucose and electrolyte solutions other than 0.9% saline and Ringer's solutions.

Table S7. Daily total fluid inputs

	Fluid Restriction Group (N=75)		Standard Care Group (N=76)	
Variable	No. with data/ no. at risk*	Volume received for all patients at risk - mL	No. with data/ no. at risk*	Volume received for all patients at risk - mL
Total fluid input				
Day 1†	75/75	2,036 (1,069-3,925)	76/76	2,205 (1,205-3,526)
Day 2	71/71	3,234 (2,272-4,407)	74/74	3,619 (2,194-5,117)
Day 3	62/62	3,097 (2,224-3,698)	62/62	2,980 (2,466-4,460)
Day 4	54/54	2,761 (1,900-3,814)	53/53	3,215 (2,514,-3,786)
Day 5	42/42	2,911 (2,440-3,796)	44/44	2,697 (2,027-3,445)
Day 1-5‡	75/75	12,411 (5,518-17,035)	76/76	13,687 (7,163-17,082)
Total§	75 /75	18,291 (5,518-34,045)	76/76	16,970 (7,163-29,889)

All volumes presented as median (interquartile range).

Abbreviations: NA, not applicable

* No. at risk is those patients who had registered data on that day(s). Where the no. at risk is below all patients allocated to the group, this is due to death or ICU discharge.

† The first day was from the time of randomization to the next start of the specific ICU's 24-hour fluid chart and lasted median 12 (IQR, 7-17) hours in the fluid restriction group and 10 (5-16) hours in standard care group.

‡ Cumulative data for day 1 to day 5.

§ Cumulative data for the full trial period in ICU to a maximum of 90 days after randomization

Table S8. Fluids given during surgery

	Fluid Restriction Group (N=75)		Standard Care Group (N=76)	
Variable	No. with surgery/ no. at risk*	Volumes for all patients at risk - mL	No. with surgery/ no. at risk*	Volumes for all patients at risk – mL
Fluids during surgery (all fluids combined)				
Day 1†	6/75	0 (0-0)	5/76	0 (0-0)
Day 2	9/71	0 (0-0)	8/74	0 (0-0)
Day 3	6/62	0 (0-0)	2/62	0 (0-0)
Day 4	3/54	0 (0-0)	3/53	0 (0-0)
Day 5	3/42	0 (0-0)	0/44	0 (0-0)
Day 1-5‡	16/75	0 (0-0)	16/76	0 (0-0)
Total§	21/75	0 (0-400)	21/76	0 (0-265)

All volumes presented as median (interquartile range).

* No. with surgery is those patients who had surgery performed in the operating room on the given day(s). No. at risk is those patients who had registered data on that day(s). Where the no. at risk is below all patients allocated to the group, this is due to death or ICU discharge.

† The first day was from the time of randomization to the next start of the specific ICU's 24-hour fluid chart and lasted median 12 (IQR, 7-17) hours in the fluid restriction group and 10 (5-16) hours in standard care group.

‡ Cumulative data for day 1 to day 5.

§ Cumulative data for the full trial period in ICU to a maximum of 90 days after randomization

Table S9. Doses of norepinephrine

	Fluid Restriction Group (N=75)		Standard Care Group (N=76)	
Variable	No. received/ no. at risk (%)*	Highest dose for all patients at risk - µg/kg/min	No. received/ no. at risk (%)*	Highest dose for all patients at risk - µg/kg/min
Norepinephrine				
First 24 hours after randomisation				
0-3 hours	75/75 (100%)	0.25 (0.12-0.40)	75/75 (100%)	0.20 (0.12-0.30)
3-6 hours	72/74 (97%)	0.23 (0.13-0.35)	73/75 (97%)	0.20 (0.12-0.35)
6-9 hours	73/75 (97%)	0.23 (0.12-0.35)	68/73 (93%)	0.19 (0.10-0.32)
9-12 hours	64/70 (91%)	0.22 (0.10-0.35)	62/68 (91%)	0.19 (0.09-0.30)
12-24 hours	63/68 (93%)	0.24 (0.10-0.40)	58/66 (88%)	0.20 (0.07-0.32)
Day 1†	75/75 (100%)	0.30 (0.14-0.43)	76/76 (100%)	0.23 (0.12-0.35)
Day 2	65/71 (92%)	0.24 (0.12-0.40)	68/74 (92%)	0.20 (0.10-0.35)
Day 3	50/62 (81%)	0.16 (0.04-0.27)	54/62 (87%)	0.12 (0.05-0.30)
Day 4	37/54 (69%)	0.08 (0-0.17)	36/53 (68%)	0.06 (0-0.21)
Day 5	25/42 (60%)	0.06 (0-0.14)	20/44 (45%)	0 (0-0.15)
Day 1-5‡	75/75 (100%)	0.34 (0.17-0.50)	76/76 (100%)	0.30 (0.18-0.44)

All doses presented as median (interquartile range).

* No. receiving is those patients who did receive norepinephrine during the given time frame. No. at risk is those patients who had registered data on that time frame. Where the no. at risk is below all patients allocated to the group, this is due to death, ICU discharge or missing data.

† The first day was from the time of randomization to the next start of the specific ICU's 24-hour fluid chart and lasted median 12 (IQR, 7-17) hours in the fluid restriction group and 10 (5-16) hours in standard care group.

‡ Cumulative data from day 1 to day 5.

Table S10. Use of co-interventions after randomization

	Fluid Restriction Group (N=75)		Standard Care Group (N=76)	
	No. received/ No. at risk (%)	Days receiving intervention for all patients at risk	No. received/ No. at risk (%)	Days receiving intervention for all patients at risk
Renal replacement therapy	16/75 (21%)	0 (0-0)	14/76 (18%)	0 (0-0)
Mechanical ventilation	57/75 (76%)	3 (1-8)	58/76 (76%)	3 (1-8)
Glucocorticoids*	23/66 (35%)	0 (0-2)	33/71 (46%)	0 (0-4)

Days receiving intervention presented as median (interquartile range).

* A total of 14 patients (9 in the fluid restriction group and 5 in the standard care group) were co-enrolled in the ADRENAL trial (NCT01448109), in which patients were randomized to receive either masked infusion of 200 mg hydrocortisone or placebo daily in the ICU for a maximum of 7 days. Data on use of glucocorticoids are not presented for these patients. All 14 patients were randomized into the ADRENAL trial within 24 hours of being randomized in the CLASSIC trial.

Table S11. Hemodynamic variables after randomization

	Fluid Restriction Group (N=75)		Standard Care Group (N=76)	
Variable	No. with data/ total no.*	Values for patients with data	No. with data/ total no.*	Values for patients with data
Highest lactate – mmol/L				
First 24 hours after randomization				
0-3 hours	69/75	2.8 (1.8-4.5)	73/76	2.3 (1.3-4.3)
3-6 hours	66/75	2.5 (1.6-4.2)	68/76	2.2 (1.3-4.1)
6-9 hours	68/75	2.1 (1.4-3.4)	61/76	2.1 (1.4-4.0)
9-12 hours	64/75	2.0 (1.3-3.4)	59/76	1.8 (1.3-3.2)
12-24 hours	67/75	1.9 (1.2-3.3)	63/76	2.1 (1.4-3.1)
Day 2	70/75	2.0 (1.4-3.5)	71/76	2.0 (1.5-3.9)
Day 3	60/75	1.6 (1.3-2.6)	62/76	1.9 (1.3-2.6)
Day 4	52/75	1.9 (1.2-2.4)	51/76	1.6 (1.4-2.3)
Day 5	41/75	1.7 (1.4-2.7)	43/76	1.6 (1.3-2.2)
Urinary output – mL				
First 24 hours after randomization				
0-3 hours	74/75	128 (50-320)	75/76	195 (72-380)
3-6 hours	75/75	150 (65-350)	75/76	170 (90-390)
6-9 hours	75/75	180 (60-390)	74/76	145 (75-335)
9-12 hours	70/75	173 (70-320)	68/76	143 (60-305)
12-24 hours	69/75	735 (330-1,205)	66/76	873 (285-1,260)
Day 1†	75/75	785 (150-1,395)	75/76	645 (225-1,330)
Day 2	70/75	1,643 (1,060-2,895)	74/76	1,755 (745-2,775)
Day 3	60/75	1,755 (1,258-2,926)	61/76	1,870 (1,350-2,850)
Day 4	52/75	2,275 (975-2,900)	53/76	1,945 (1,055-3,750)
Day 5	42/75	2,450 (1,060-3,100)	43/76	2,540 (1,450-2,820)
Lowest MAP – mmHg				
First 24 hours after randomization				
0-3 hours	74/75	65 (60-69)	75/76	65 (58-70)
3-6 hours	74/75	66 (60-71)	76/76	64 (59-70)
6-9 hours	75/75	65 (60-72)	74/76	66 (60-70)
9-12 hours	70/75	66 (60-70)	68/76	65 (60-71)
12-24 hours	69/75	64 (59-66)	66/76	64 (58-67)
Day 1†	75/75	62 (55-66)	76/76	62 (55-67)

Day 2	71/75	62 (55-66)	74/76	62 (55-66)
Day 3	62/75	65 (60-70)	61/76	62 (60-66)
Day 4	54/75	65 (60-70)	53/76	65 (59-71)
Day 5	42/75	65 (59-68)	44/76	66 (62-74)
Highest heart rate – beats/min				
First 24 hours after randomization				
0-3 hours	75/75	104 (90-118)	75/76	105 (90-123)
3-6 hours	75/75	101 (86-117)	76/76	102 (89-121)
6-9 hours	75/75	100 (90-115)	74/76	101 (92-117)
9-12 hours	70/75	102 (90-117)	68/76	102 (85-115)
12-24 hours	69/75	111 (98-125)	66/76	105 (95-121)

All volumes presented as median (interquartile range).

Abbreviations: MAP, mean arterial pressure.

SI conversion factors: to convert plasma lactate from mmol/L to mg/dL divide by 0.111.

* No. with data is those patients who had registered data in that time frame. Where the no. with data is below all patients allocated to the group, this is due to death, ICU discharge or missing data.

† The first day was from the time of randomization to the next start of the specific ICU's 24-hour fluid chart and lasted median 12 (IQR, 7-17) hours in the fluid restriction group and 10 (5-16) hours in standard care group.

Table S12. The co-primary outcomes adjusted for stratification variable and baseline covariates

	Fluid Restriction Group (N=75)	Standard Care Group (N=76)	Restrictive vs. Standard Care (95% CI)* - mL	P-value†
	Estimated mean - mL	Estimated mean - mL		
Volume of resuscitation fluid first 5 days after randomization				
Adjustment for stratification variable‡	1,687	2,928	-1,241 (-2,043 to -439)	0.003
Adjustment for stratification and baseline covariates§	1,648	2,982	-1,334 (-2,161 to -507)	0.002
Volume of resuscitation fluid during ICU stay				
Adjustment for stratification variable‡	1,992	3,399	-1,407 (-2,358 to -456)	0.004
Adjustment for stratification and baseline covariates§	1,953	3,484	-1,531 (-2,512 to -549)	0.002

Abbreviations: CI, confidence interval.

* Estimated mean of the restrictive group minus estimated mean of the standard care group.

† P of generalized linear model.

‡ Adjustment by trial site (the primary analysis)

§ Adjustment by trial site and the following baseline covariates: (1) age/years at randomization, (2) estimated weight/kg at randomization, (3) highest dose of norepinephrine in the 24 hours prior to randomization, (4) surgery during current hospitalization but prior to randomization Y/N and (5) more than 5 L of fluid given in the 24 hours prior to randomization Y/N. One or more baseline variables for adjustment were missing for 2 (3%) and 3 (4%) patients in the fluid restriction and standard care group, respectively. Complete case analyses are presented.

Table S13. Per-protocol analyses for the co-primary outcome measures

Outcome	Standard Care Group (N=53)* Fluid Restriction Group (N=41)*	Mean	Percentiles					P-value
			10%	25%	50%	75%	90%	
Co-primary outcome measures								
Volumes of resuscitation fluid (ml)								
First 5 days after randomization	Standard Care	2,460	250	500	1,500	3,000	6,500	
	Fluid Restriction	636	0	0	481	500	1,000	<0.001†
During ICU stay	Standard Care	2,810	250	750	1,500	3,500	6,750	
	Fluid Restriction	642	0	0	481	500	1,000	<0.001†

* See Table 1 for detailed description of eligible/non-eligible patients.

† P of the non-parametric van Elteren test adjusted by trial site (stratification variable).

Table S14. Serious adverse reactions

	Fluid Restriction group (N=75)		Standard Care group (N=76)	
Variable	No. with event/ No. at risk (%)*	Days with event for all patients at risk	No. with event/ No. at risk (%)*	Days with event for all patients at risk
Serious adverse reactions to isotonic crystalloids – 0.9% Saline and Ringer's solutions				
Hypernatremia†	6/75 (8%)	0 (0-0)	8/76 (11%)	0 (0-0)
Hyperchloremic acidosis‡	23/75 (31%)	0 (0-1)	23/76 (30%)	0 (0-1)
Seizures§	0/75 (0%)	0 (0-0)	2/76 (3%)	0 (0-0)
Central pontine myelinolysis‖	0/75 (0%)	0 (0-0)	0/76 (0%)	0 (0-0)
Anaphylactic reaction¶	0/75 (0%)	0 (0-0)	0/76 (0%)	0 (0-0)
Serious adverse reactions to norepinephrine				
Cardiac arrhythmia**				
Ventricular tachyarrhythmia	0/75 (0%)	0 (0-0)	3/76 (4%)	0 (0-0)
Supraventricular tachyarrhythmia	23/75 (31%)	3 (2-6)	24/76 (32%)	3 (1-4)
Delirium††	13/75 (17%)	0 (0-0)	11/76 (14%)	0 (0-0)
Intracerebral hemorrhage‡‡	0/75 (0%)	0 (0-0)	1/76 (1%)	0 (0-0)

Days with event are presented as median (interquartile range).
Investigators were asked daily to register events of serious adverse reactions. A suspected or confirmed association between the event and the drug was not required.

* No. receiving is those patients who did receive the specific solution on the given day(s). No. at risk is those patients who were allocated to the intervention group.

† Hypernatriemia defined as plasma sodium (p-Na) above 155 mmol/L.

‡ Hyperchloremic acidosis[c] defined as pH below 7.35 AND plasma chloride above 110 mmol/L.

§ Clinical signs of seizures OR seizures verified by electroencephalogram.

‖ Verified by CT or MR scan.

¶ Anaphylactic reaction defined as urticaria and at least one of the following: Worsened circulation (>20% decrease in blood pressure or >20% increase in vasopressor dose), increased airway resistance (>20 % increase in peak pressure on the ventilator), clinical stridor or bronchospasm, OR subsequent treatment with broncodilators.

** Cardiac arrhythmia resulting in intervention (drugs or electrical cardioversion) against it.

†† Psychiatric symptoms (delirium, hallucinations, delusions) resulting in medication.

‡‡ Verified by CT or MR scan.

Table S15. Ischemic events in the ICU after randomization

	Fluid Restriction Group (N=75)	Standard Care Group (N=76)
	No. with ischemia/ no. at risk (%)	No. with ischemia/ no. at risk (%)
Any ischemic event	3/75 (4%)	9/76 (12%)
Limb ischemia*	0/75 (0%)	2/76 (3%)
Intestinal ischemia†	2/75 (3%)	5/76 (7%)
Myocardial ischemia‡	1/75 (1%)	1/76 (1%)
Cerebral ischemia§	0/75 (0%)	2/76 (3%)

One patient in the standard care group had ischemia at 2 anatomic sites.

* Limb ischemia: clinical signs and at least one of the following: initiation/increased antithrombotic treatment, open/percutaneous intervention OR amputation

† Intestinal ischemia verified by endoscopy or open surgery.

‡ Myocardial ischemia: diagnosis of acute myocardial infarction (ST-elevation myocardial infarction or non-ST-elevation myocardial infarction) or unstable angina pectoris, according to the criteria in the clinical setting in question (elevated biomarkers, ischemic signs on electrocardiogram and/or clinical presentation).

§ Cerebral ischemia verified by CT or MR scan.

Table S16. Analyses of combined input of non-resuscitation fluids

Outcome	Fluid restriction group (n=75)	Standard care group (n=76)	Fluid restriction vs. standard care (95% CI)*	P value
Volumes of non-resuscitation fluid (mL)				
First 5 days after randomization	11,083 (5,518-14,728) [10,090]	10,687 (5,304-12,929) [9,668]	422 (-1,402 to 2,245)	0.63†
During ICU stay after randomization	16,116 (5,518-30,970) [19,466]	13,408 (5,482-23,306) [20,095]	-629 (-9,220 to 7,962)	0.92†

Values in the two intervention groups are presented as medians (interquartile ranges) [estimated mean values adjusted for trial site]. Non-resuscitation fluids included nutrition, fluids with medication, crystalloids and colloids on non-resuscitation indication, and blood products.

Abbreviations: CI, confidence interval.

A total of 33 patients (8 had died and 25 had been discharged) and 32 (7 had died and 25 had been discharged) were not in the ICU on day 5 in the fluid restriction group and the standard care group, respectively. The ICU length of stay was median 6 days (IQR 3-11) and 5 (3-10) in the fluid restriction group and the standard care group, respectively.

* Estimated mean of the restrictive group minus estimated mean of the standard care group.

† Non-parametric p-values. The estimated differences are presented where applicable even though the assumptions for parametric testing were not fully met.

Figure 4. Recruitment over time

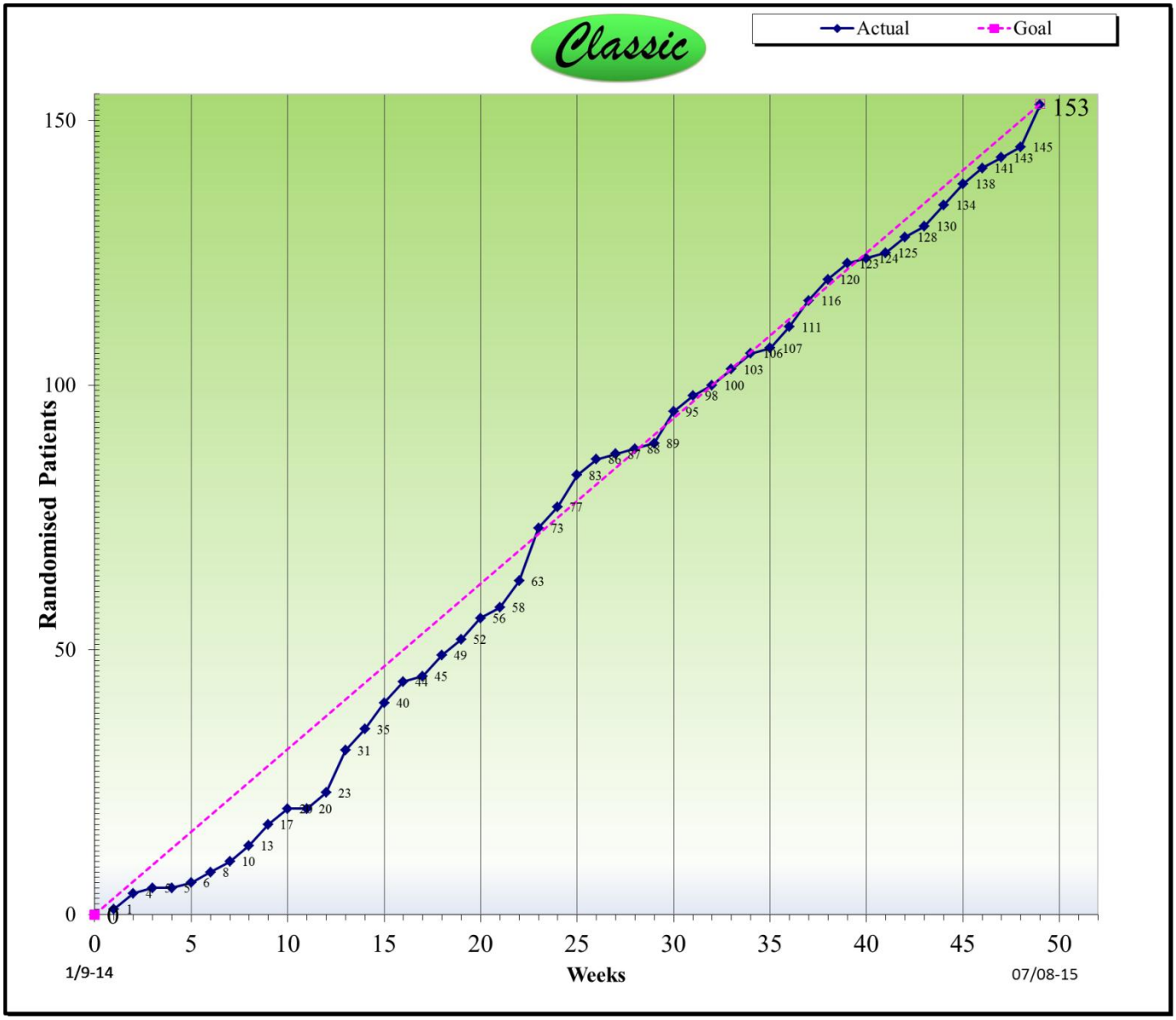


Figure 5. Percentiles of total fluid input given after randomization.

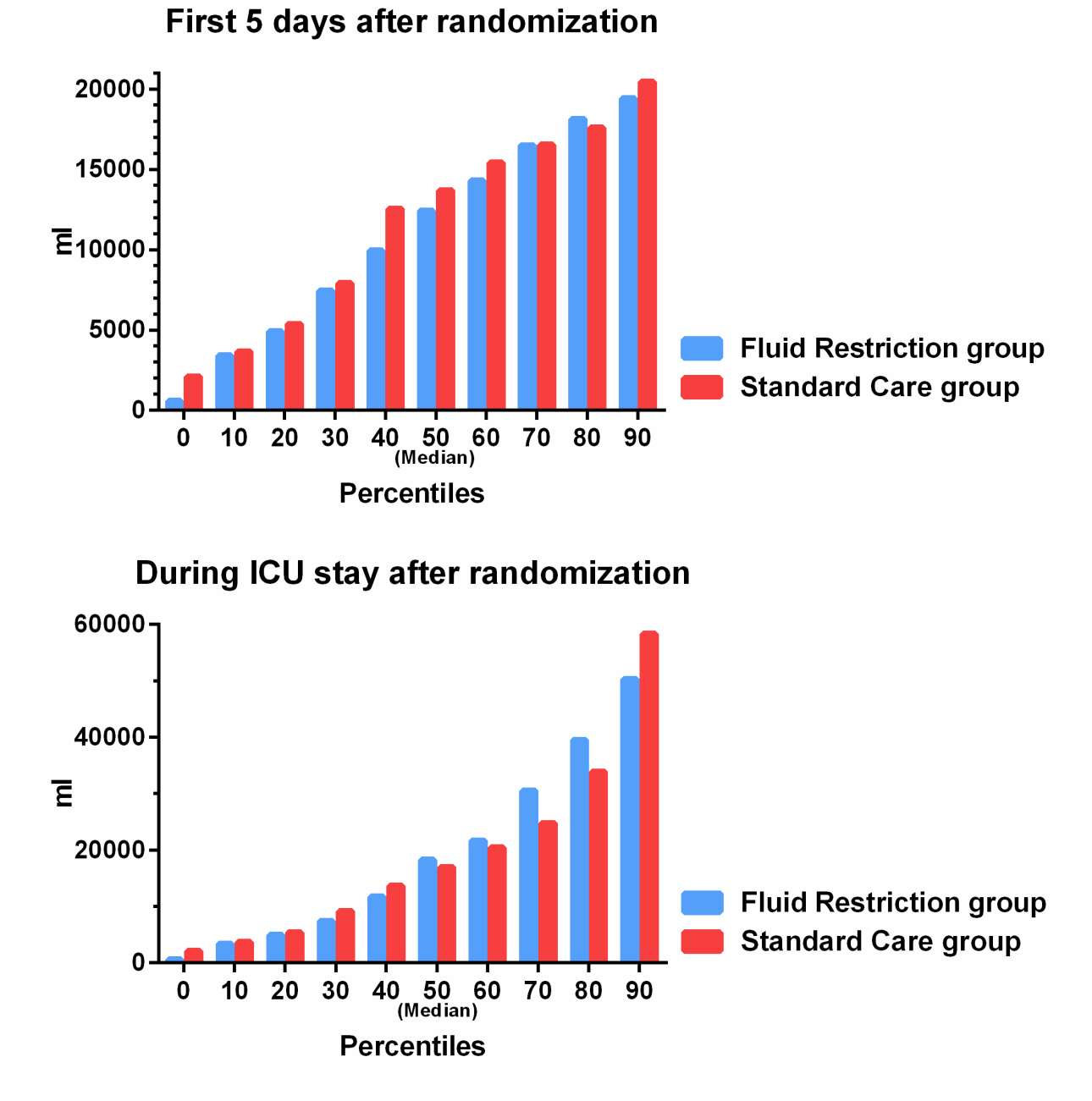


Figure 6. Percentiles of cumulated fluid balances after randomization.

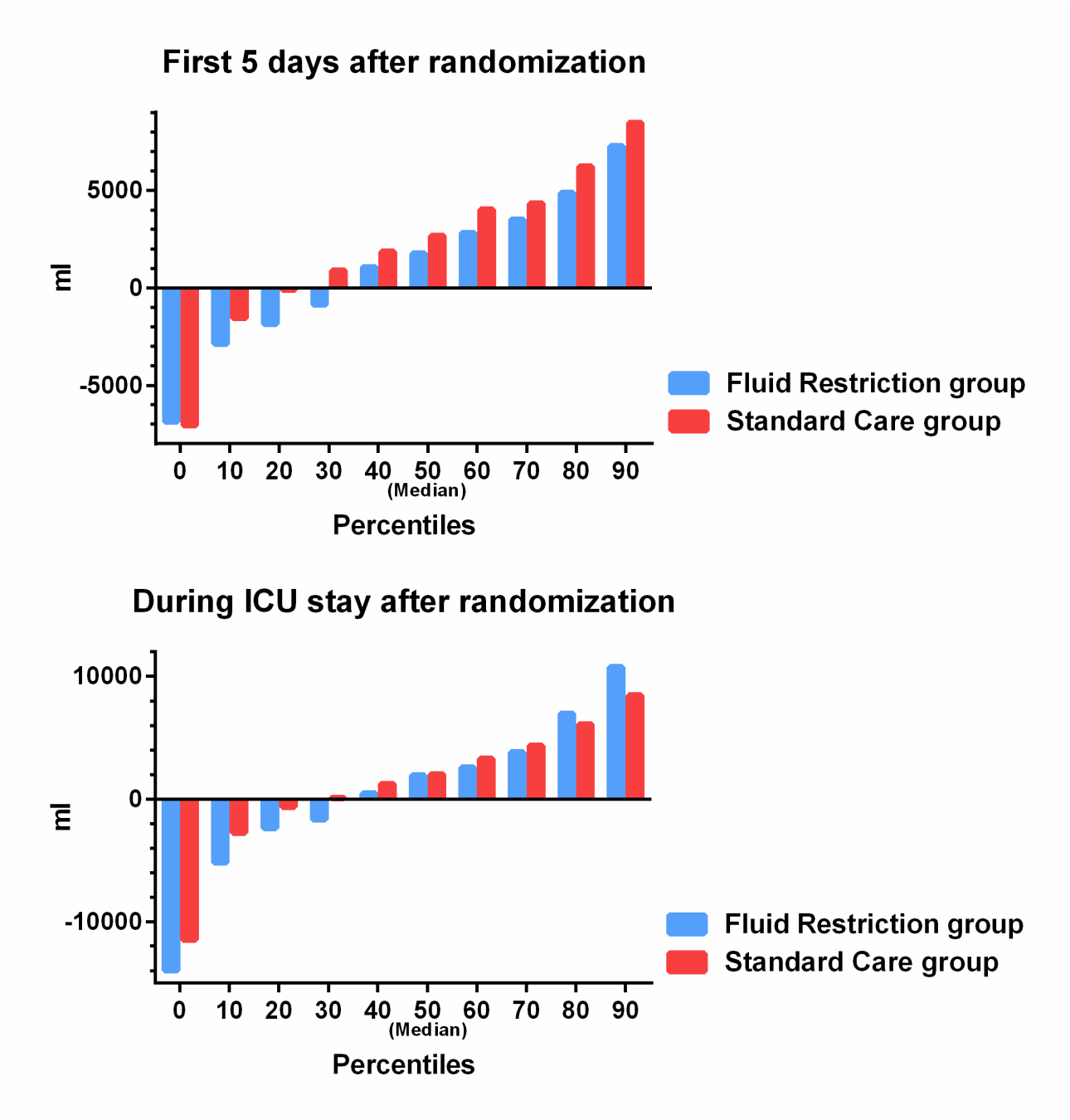
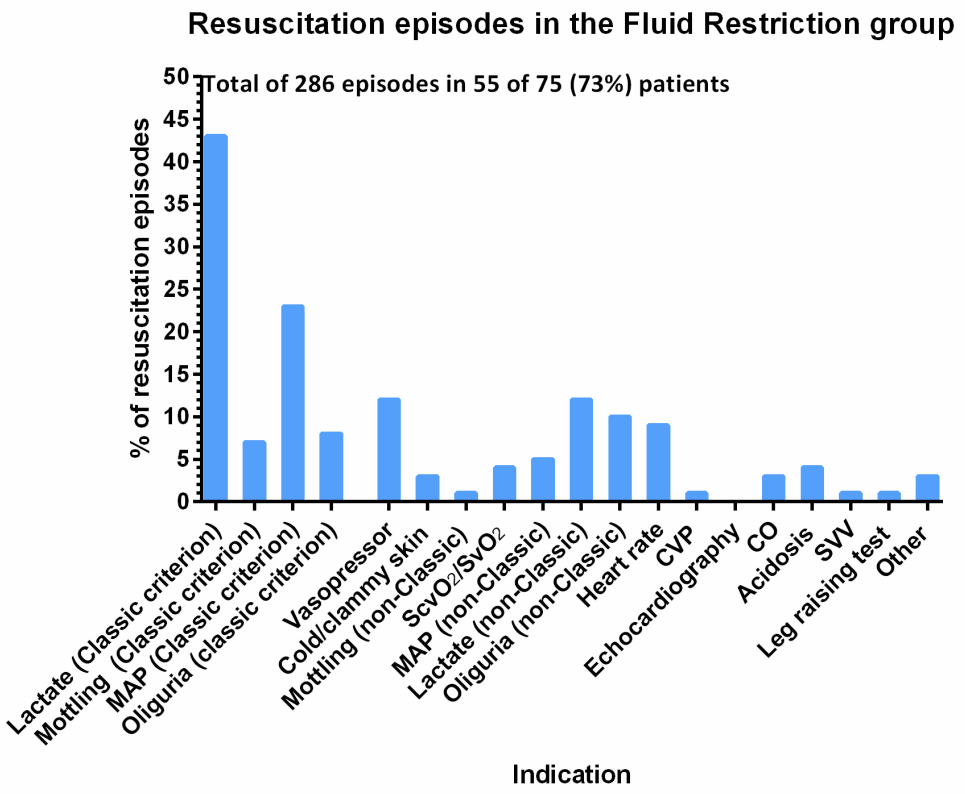
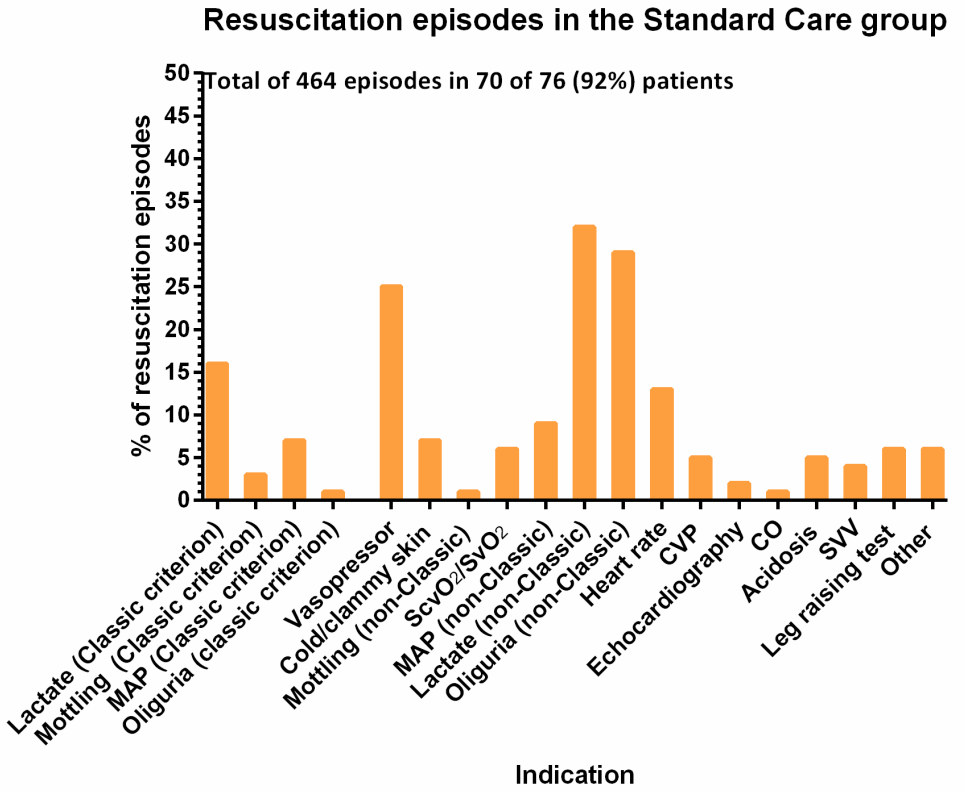


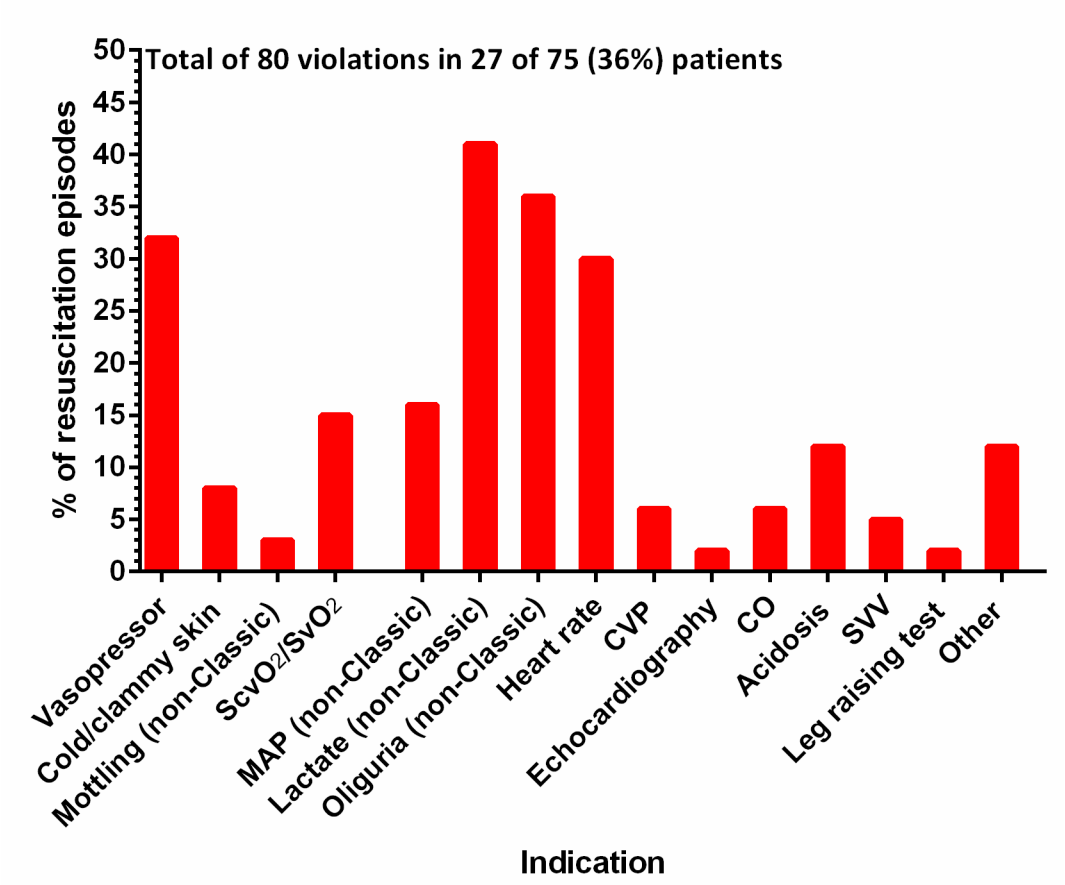
Figure 7. Indications for fluid resuscitation episodes.





Indications given by the clinicians for fluid resuscitation episodes after randomization. More than one indication could be given.
Non-Classic criteria:
Mottling (non-classic criterion): Mottling present, but not beyond the edge of the kneecap.
MAP (non-classic criterion): Low MAP, but above 50 mmHg.
Lactate (non-classic criterion): Elevated lactate, but below 4 mmol/l.
Oliguria (non-classic criterion): Low urinary output, but above 0.1 ml/kg/hour AND/OR later than 2 hours after randomization.
Abbreviations: $ScvO_2$, central venous oxygen saturation. SvO_2, mixed venous oxygen saturation. MAP, mean arterial pressure. CVP, central venous pressure. CO, cardiac output. SVV, stroke volume variation.

Figure 8. Indications for fluid resuscitation violating the protocol in the fluid restriction group



Indications given by the clinicians for fluid resuscitation episodes violating the protocol in the fluid restriction group – more than one indication could be given.

Abbreviations: $ScvO_2$, central venous oxygen saturation. SvO_2, mixed venous oxygen saturation. MAP, mean arterial pressure. CVP, central venous pressure. CO, cardiac output. SVV, stroke volume variation.

Non-Classic criteria violations:

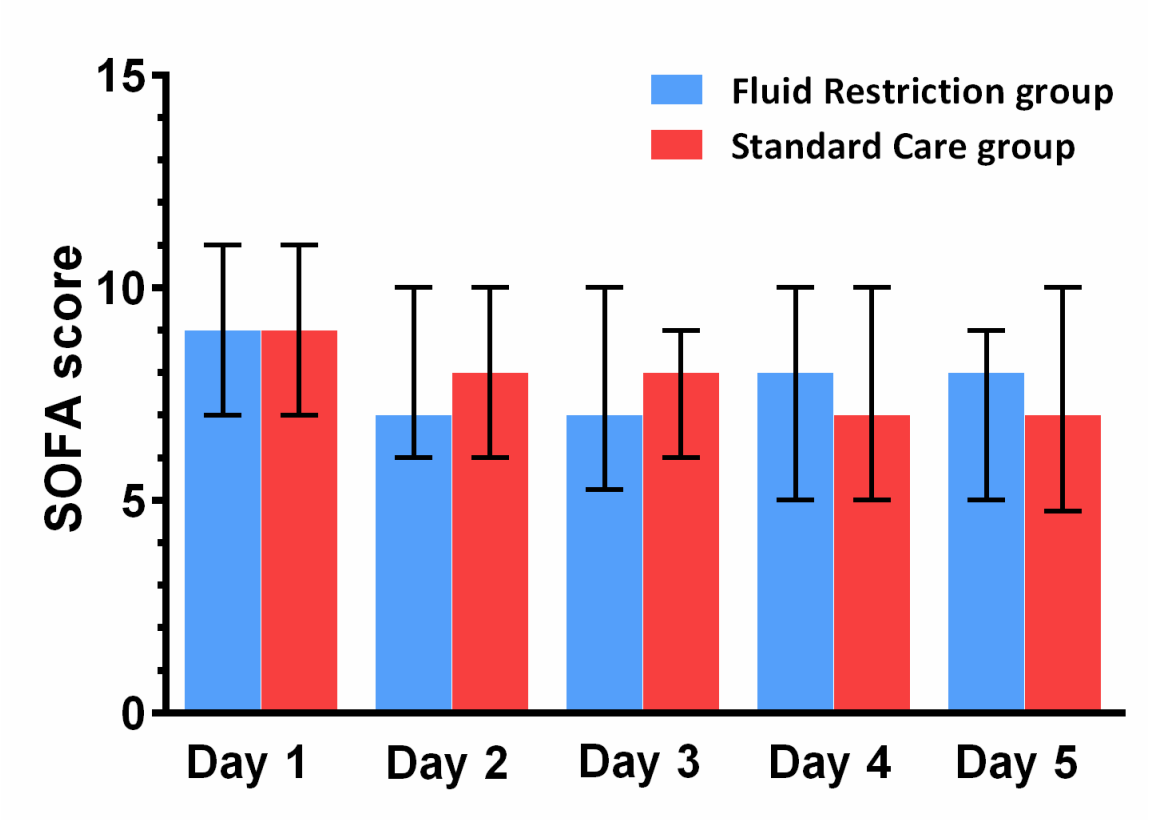
Mottling (non-classic criterion) violation: Mottling present, but not extending beyond the edge of the kneecap.

MAP (non-classic criterion) violation: Low MAP, but above 50 mmHg.

Lactate (non-classic criterion) violation: Elevated lactate, but below 4 mmol/l.

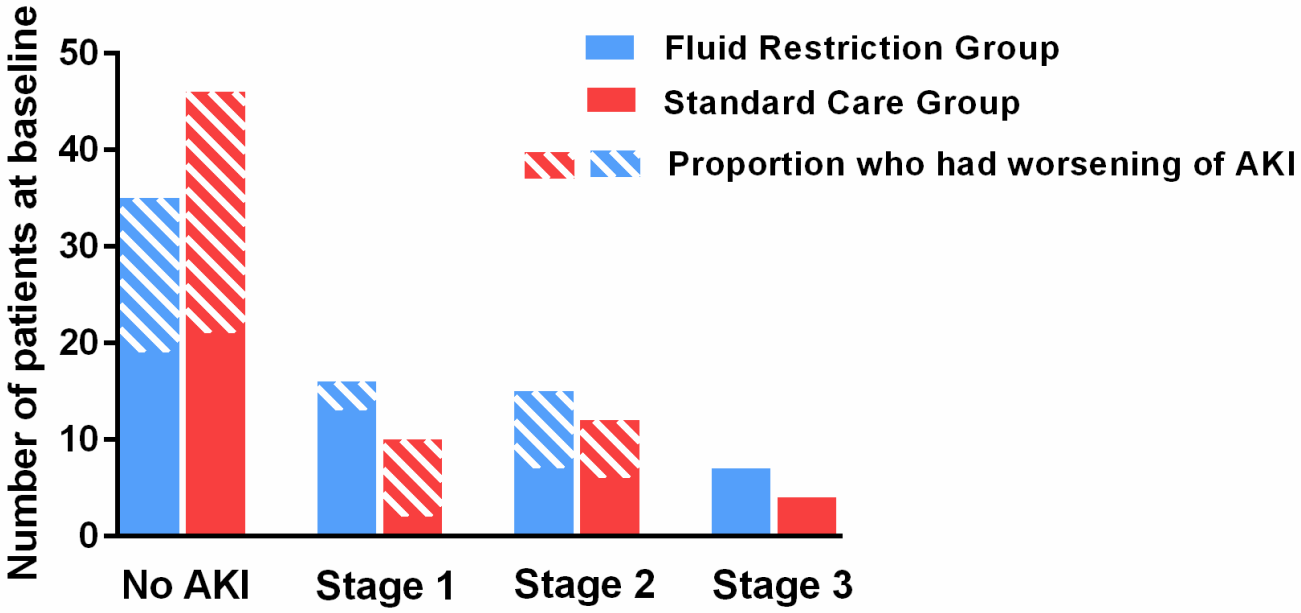
Oliguria (non-classic criterion) violation: Low urinary output, but above 0.1 ml/kg/hour AND/OR later than 2 hours after randomization.

Figure 9. Daily SOFA scores (excl. the GCS subscore) after randomization.



Daily SOFA score (excluding. the GCS score subscore) presented as medians with interquartile ranges (error bars). Day 1 was from the time of randomization to the next start of the specific ICU's 24-hour fluid chart and lasted median 12 (IQR, 7-17) hours in the fluid restriction group and 10 (5-16) hours in standard care group. In case of missing data on one or more of the subscores relying on blood sampling last observation carried forward was performed.

Figure 10. Baseline KDIGO and subsequent worsening of AKI.



Patients are stratified by KDIGO stage prior to randomisation. Each column consists of number patients who did not have worsening of AKI (solid) and the number of patients who did have worsening of AKI (white stripes). A total of 6 patients (4%) had either missing baseline plasma creatinine or did not have any plasma creatinine measurements during ICU stay – these patients were not included in the figure. Abbreviations: AKI, acute kidney injury.

References

1. Group KAKIW. KDIGO Clinical Practice Guideline for Acute Kidney Injury. *Kidney International Supplements.* 2012;2(1):1-138.
2. Bone RC, Balk RA, Cerra FB, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest.* 1992;101(6):1644-1655.
3. Dellinger RP, Levy MM, Rhodes A, et al. Surviving sepsis campaign: International guidelines for management of severe sepsis and septic shock, 2012. *Intensive Care Med.* 2013;39(2):165-228.

CLASSIC Trial Supplement 2

Conservative vs. Liberal Approach to fluid therapy of Septic Shock in Intensive Care (Classic) – a randomised clinical trial

Protocol version 4.3 September 25th 2014
In drafting of present protocol Copenhagen Trial Unit's Standard Operating Procedures were used.

Applicable protocol registration numbers (ClinicalTrials.gov: NCT02079402, Danish Ethics committee identifier: H-3-2014-057, EudraCT identifier: 2014-000902-37, Danish Data Protection Agency identifier: Danish Health and Health Medicine Authority identifier: 2014043517)
Application for governance approvals have been submitted for The Health Research Ethics Committee, Capital Region of Denmark, The Danish Data Protection Agency and the Danish Health Medicine Authority.

Amendment requiring new protocol version

Change: The definition of circulatory impairment in the inclusion criterion "Suspected or confirmed circulatory impairment (hypotension/hypoperfusion/hypovolemia) for no more than 12 hours including the hours preceding ICU admission" was revised.
Previous protocol version: 4.2 June 4th 2014
New (final) protocol version: 4.3 September 25th 2014
Applied for: September 25th 2014
Approved by Ethics Committee and Medicines Agency and effectuated: October 30th 2014
Changed from: Systolic blood pressure < 90 mmHg, heart rate > 110 beats/min, cold and clammy skin, lactate > 2 mmol/l, oliguria, patient judged as being hypovolemic, skin mottling OR weak peripheral pulses.
Changed to: Systolic blood pressure < 90 mmHg, heart rate > 140 beats/min, lactate ≥ 4 mmol/l, OR use of vasopressors.
Reasons for change: More patients - who were otherwise relevant to enroll - had circulatory impairment for more than 12 hours according to the previous definition than expected. Furthermore, we wished to simplify the definition as it proved a challenge to achieve valid data from patient records on some of the previously used criteria (e.g. 'cold and clammy skin', 'patient judged to be hypovolemic', 'skin mottling' and 'weak peripheral pulses'.)

Amendments not requiring new protocol version

Action: Promotion of a secondary outcome to a co-primary outcome.
Applied for: June 19th 2015
Approved by Ethics Committee and Medicines Agency: July 31st 2015
Outcome: Amount of resuscitation fluid during ICU stay
Reasons for action:
We originally defined the primary outcome as volume of resuscitation fluid administered within first 5 days. Before the data were available for analyses, we decided after careful consideration to promote volume of resuscitation fluid during entire ICU stay to a co-primary outcome from a secondary outcome. We decided this for the following reasons: First, the intervention period was entire ICU stay and we considered it pertinent for this to be reflected in the primary outcome. Second, for some patients, septic shock may not be resolved after 5 days and some patients experience a "second hit" of inflammation facilitating further fluid resuscitation beyond 5 days. If an effect of reduced fluid resuscitation volume on day 5 was compensated by increased fluid resuscitation volume during the remaining ICU stay, this would have been important information. Whether such a finding, would affect patient-centred outcomes is uncertain, but it would be important when assessing the effect of the protocol and when designing a large-scale trial. In order to account for multiplicity issues (increased risk of type 1 error) when using two co-primary outcome measures we adjusted the level of significance. Because we expected a degree of correlation between the two outcomes, we chose to adjust the level of significance by a factor in between a full Bonferroni adjustment and no adjustment at all, that is 0.05/1.5=0.033 (see Statistical analysis plan for details).

Action: Specifying that the observational period for use of renal replacement therapy was 90 days following randomization for the outcome 'Worsening of acute kidney injury according to KDIGO criteria during ICU stay'.
Applied for: December 8th 2015
Approved by Ethics Committee and Medicines Agency: December 23rd 2015
Reasons for action: Creatinine was assessed daily during ICU stay. Use of renal replacement therapy, however, was assessed daily during the entire 90 day follow-up period for each enrolled patient. We decided to include use of renal replacement therapy after ICU discharge, because it may reflect worsening of kidney function during the intervention period.

Abstract

Background

Fluid resuscitation is a key intervention in treatment of sepsis, but exact indications for fluid and amount of fluid administered are not established. Current guidelines for fluid therapy beyond 6 hours are vague and ungraded and most observational studies suggest harm with increasing positive fluid balance.

Objective

To assess feasibility of a protocol comparing a conservative (trigger guided) vs. liberal (target guided) approach to fluid resuscitation in patients with septic shock after initial fluid resuscitation.

Design

Multicentre, parallel group, centrally randomised, open label trial with adequate generation of allocation sequence, and adequate allocation concealment.

Inclusion criteria

- Adult intensive care patients (age ≥ 18 years) with sepsis defined as 2 of 4 SIRS criteria fulfilled within 24 hours and suspected or confirmed site of infection or positive blood culture

AND

- Suspected or confirmed circulatory impairment (hypotension/hypoperfusion/hypovolemia) for no more than 12 hours including the hours preceding ICU admission

AND

- At least 30 ml/kg IBW fluid (colloids, crystalloids or blood products) given in the last 6 hours

AND

- Shock (defined as ongoing infusion of noradrenaline (any dose) to maintain blood pressure)

Exclusion criteria:

Use of any form of renal replacement therapy (RRT) or RRT deemed imminent by the ICU doctor, i.e. RRT will be initiated within 6 hours, severe hyperkalemia (p-K > 6 mM), plasma creatinine > 350 µmol/l, invasively ventilated with FiO_2 > 0.80 and PEEP > 10 cmH_2O, life-threatening bleeding, kidney or liver transplant during current admission, burns > 10% BSA, previously enrolled in the CLASSIC trial and has finished the 90 day observation period, patients for whom it has been decided not to give full life support including mechanical ventilation and RRT OR consent not obtainable.

Interventions
For all included patients: Noradrenaline administration targeting MAP 65 mmHg. Patients randomised to receive:

1. Conservative (trigger guided) fluid resuscitation.

250-500ml of isotonic crystalloid solution may be given followed by re-evaluation if plasma lactate ≥ 4 mmol/l OR severe hypotension (MAP < 50mmg) OR mottling beyond edge of kneecap OR severe oliguria (only in the first two hours after randomisation).

2. Liberal (target guided) fluid resuscitation.

Fluid bolus of isotonic crystalloid solution may be given as long as hemodynamic variables improve (hemodynamic variable(s) of choice).

Primary outcome:

1. Amount of resuscitation fluid given in the first 5 days after randomisation

Secondary outcomes:

2.1 Amount of resuscitation fluid given during ICU stay
2.2 Fluid balance and total fluid input at day 5 after randomisation and during ICU stay
2.3 Number of patients with major protocol violations
2.4 Accumulated serious adverse reactions (SARs) in ICU

Exploratory outcomes:

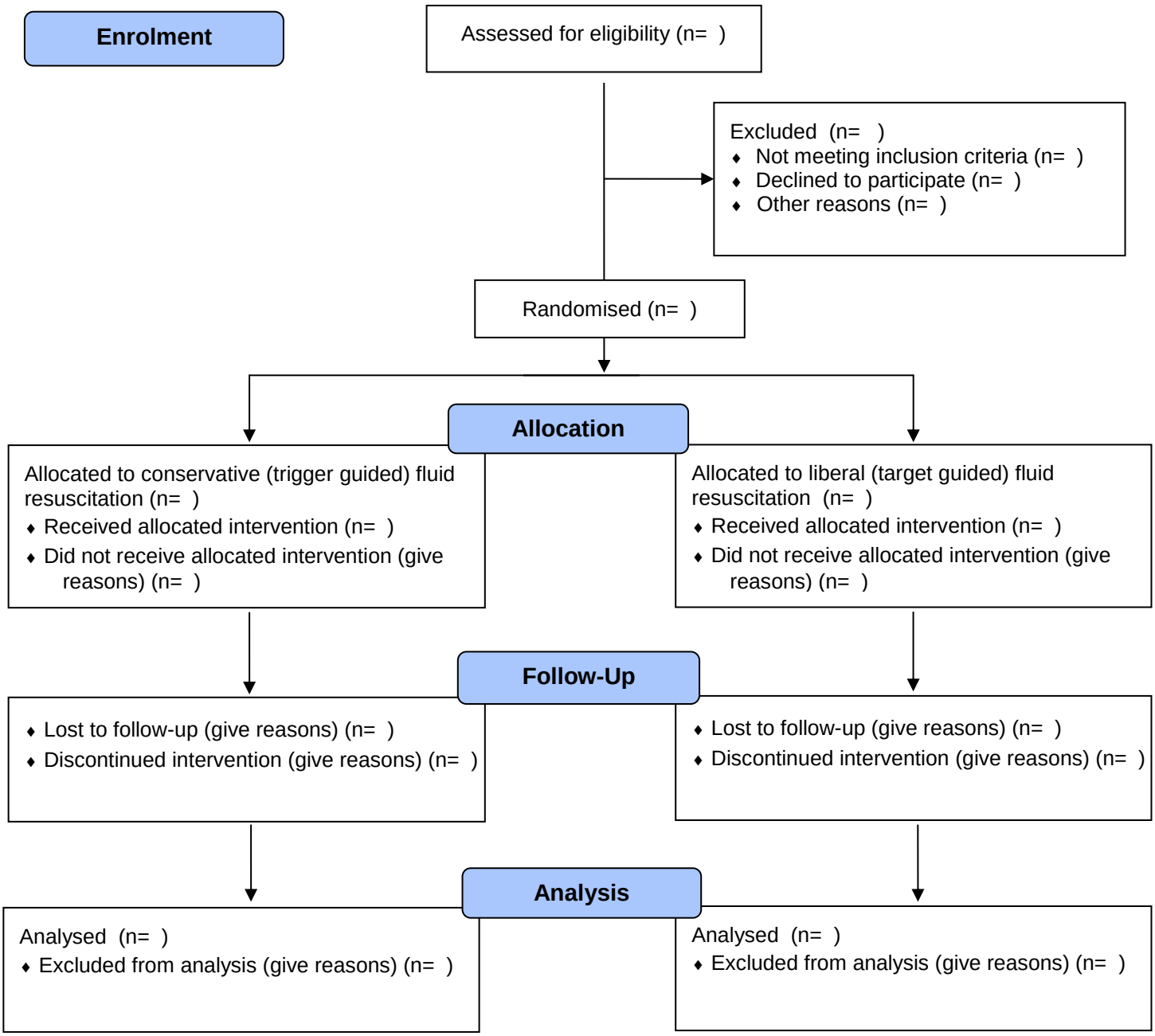
a. All-cause mortality at day 90
b. Mortality during the total observation time
c. Days alive without use of mechanical ventilation
d. Days alive without use of RRT
e. Worsening of acute kidney injury according to KDIGO criteria during ICU stay
f. Delta-creatinine (defined as highest p-creatinine during ICU stay minus most recent p-creatinine prior to randomisation)
g. Ischaemic events in ICU.

Trial size:
150 randomised patients in multiple ICUs

Time schedule:
Enrolment is planned to start May 2014

Trial flow chart

The flowchart (n=) will be filled in during or at the end of the trial.



Administrative information

Sponsor

Name, address, telephone number, and e-mail.

Trial sites

TBD

Investigators

TBD

Steering Committee

Management committee:

Anders Perner, Peter Buhl Hjortrup and Nicolai Haase, Dept. of Intensive Care, Copenhagen University Hospital, Rigshospitalet, Denmark.
Jørn Wetterslev, Copenhagen Trial Unit, Copenhagen University Hospital, Rigshospitalet, Denmark.
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Lars Nebrich/Carsten Albeck, Dept. of Intensive Care, Hvidovre Hospital, Denmark
David Lodahl, Dept. og Intensive Care, Holstebro Hospital, Denmark.

Signatures

Insert when relevant.

Table of contents

List of abbreviations

AKI – acute kidney injury
ALI – acute lung injury
BSA – body surface area
CO – cardiac output
eCRF – electronic case report form
EGDT – early goal directed therapy
FiO_2 – Fraction of inspired oxygen
IBW – ideal body weight
ICU – intensive care unit
IV – intravenous
LOS – length of stay
MAP – mean arterial pressure
PEEP – Positive end-expiratory pressure
RBC – red blood cells
RCT – randomised clinical trial
RRT – renal replacement therapy
$ScvO_2$ – central venous oxygen saturation
SAE – serious adverse event
SAR – serious adverse reaction
SUSAR – suspected unexpected serious adverse reaction
SIRS – systemic inflammation response syndrome
SOFA – sequential organ failure assessment
SAPS II – simplified acute physiology score II
SSC – surviving sepsis campaign
SV – stroke volume

1. Introduction and background

1.1 The patient population

Sepsis is characterised by inflammation-induced endothelial dysfunction in response to infection leading to vascular leakage and vasodilatation[1,2]. Sepsis may lead to relative and absolute hypovolaemia, organ hypoperfusion and shock. In severe sepsis progressive multiple organ failure may occur with mortality rates close to 50%[3].
Sepsis is one of the leading causes of death worldwide and may account for 9% of all deaths in high-income countries[4]. Severe sepsis has a population incidence of approximately 0.5/1000 in high-income countries accounting for approximately 10% of all ICU admission[5]. Improved treatment of sepsis may thus have impact on global health and health care costs.

1.2 Current practice

Treatment of severe sepsis is complex with multiple concomitant interventions[6]. Fluid resuscitation is a key intervention, but it is not established how to give the correct amount. Current Surviving Sepsis Campaign guidelines for resuscitation in the first 6 hours are based on the protocol described by Rivers et al[7], but beyond 6 hours the guidance for continued fluid resuscitation is vague and ungraded[6].

1.3 Trial interventions

Fluid therapy beyond 6 hours:

1. Conservative (trigger guided) fluid resuscitation.
2. Liberal (target guided) fluid resuscitation.

1.3.1 Clinical data on fluid resuscitation in sepsis

A randomised clinical trial investigating amount of fluid administration in resuscitation of sepsis beyond 6 hours has not been conducted.

Current Surviving Sepsis Campaign (SSC) guidelines for resuscitation in the first 6 hours are based on the protocol of Early Goal Directed Therapy (EGDT) described by Rivers et al[7]. This was a single centre trial investigating the effect of targeted resuscitation in patients with severe sepsis or septic shock in the first 6 hours after admittance to an emergency department in the US. 263 septic shock patients were randomised to complex protocols. Fluid administration was in both arms to be guided by central venous pressure (target 8-12 mmHg), and the difference between the two interventions was use of $ScvO_2$ to guide inotropes and blood transfusions in the EGDT arm. Nevertheless patients randomised to the EGDT arm received significantly more fluid during the first 6 hours (mean 5.0 L vs. 3.5 L, $p<0.001$) received significantly less fluid during following 66 hours (mean 8.6 L vs. 10.6 L, $p=0.01$) and had significantly decreased hospital mortality (30.5% vs. 46.5%, $p=0.009$). Since the latest update of the SSC guidelines, a large RCT on 1341 patients with septic shock randomised to either EGDT (Rivers protocol), protocol based standard therapy or usual care has been published.

The study found no difference in 60-day in-hospital mortality between patients randomised to EGDT vs. usual care (21.0% vs. 18.9%, non-significant). The patients were less sick (mean baseline lactate ≈5 vs. ≈7 mmol/l) and received less fluids (mean 2.8 L and 2.3 L vs. 5.0 L and 3.5 L first six hours for EGDT and usual care, respectively) compared to patients in the Rivers study.[8] Currently two other large scale multicentre trials (ARISE (NCT00975793) and PROMISE (ISRCTN36307479)) are testing the EGDT protocol vs. standard care.

Beyond 6 hours the guidance for continued fluid resuscitation is vague and ungraded. The SSC recommendation for fluid resuscitation beyond 6 hours states: "We recommend that a fluid challenge technique be applied wherein fluid administration is continued as long as there is hemodynamic improvement either based on dynamic (e.g., change in pulse pressure, stroke volume variation) or static (e.g., arterial pressure, heart rate) variables."[6]

An RCT including 1001 patients with acute lung injury comparing complex protocols of conservative vs. liberal fluid management has been published[9]. Patients receiving liberal fluid management received more fluid, less diuretics and had more positive fluid balance (7.0 L vs. –0.1 L. p<0.001). There were no statistical significant difference in 60-day mortality between the two groups, but the conservative group had shortened duration on mechanical ventilation and improved lung function without increasing non-pulmonary failures. The included patients were highly selected and the protocol was instituted late (mean 42 hours after ICU admission).
An RCT including 3141 African children with severe infection and impaired perfusion comparing saline-bolus infusion, albumin-bolus and no bolus has been published[10]. All included patients received maintenance fluid (2.5-4.0 ml/kg/h). The trial was stopped early because of a surprising increase in 48-hour mortality in patients receiving bolus (both the saline and albumin groups combined) vs. no bolus with relative risk of death 1.45 (95% CI 1.13-1.86, p=0.003). The trial was performed in sub-Saharan Africa with high incidence of malaria (57%) and limited health care resources.

Observational studies in sepsis show conflicting results. A retrospective study of 778 patients with septic shock reported increased 28-day mortality with increased fluid balance after both 12 hours and 4 days, remaining significant after adjusting for age and APACHE II score[11]. In an observational sub-study of 1,120 septic patients with acute renal failure higher mean daily fluid balance in a multivariate cox regression analysis was associated with increased 60-day mortality[12]**.** A retrospective cohort study of 212 patients with septic shock showed decrease in hospital mortality in patients achieving 'adequate initial fluid resuscitation' (defined as the administration of an initial fluid bolus of ≥ 20 mL/kg prior to and achievement of a central venous pressure of ≥ 8 mm Hg within 6 h after the onset of therapy with vasopressors) and 'conservative late fluid management' (defined as even-to-negative fluid balance measured on at least 2 consecutive days during the first 7 days after septic shock onset)[13]. In contrast, a prospective observational study of 164 patients with septic shock reported that higher fluid volumes given during first three days of shock was associated with improved survival[14].

Taken together, the observational studies suggest harm with increasing fluid balance, but conclusions about causality must be drawn with caution due to the inherent bias in observational studies.

1.4 Risks and benefits

A conservative approach to fluid therapy after initial fluid resuscitation may be beneficial in lessening tissue edema including pulmonary edema and shortening the duration of mechanical ventilation, but it may also compromise peripheral and/or organ perfusion.

1.5 Ethical justification and trial rationale

Fluid resuscitation is a key intervention in severe sepsis and septic shock, but the optimal amount of fluid to be given has not been established. Observational studies show conflicting results with most indicating possible harm with increasing positive fluid balance. As the intervention is very frequent, but not evidence based and carries potential risks we consider it to be in great interest for society and patients to perform research in this area. The present trial is a feasibility trial, i.e. a trial assessing the feasibility of the proposed protocol in a clinical setting. Should the trial prove feasible with separation between the two interventions, a large scale trial assessing patient important outcomes is intended. Thus it is the opinion of the steering committee that this study is of great interest and ethically justified.
Patients will only be enrolled after informed consent, but fluid therapy is time-dependent and many patients will be unconscious; thus patients will be included after proxy consent (physician and/or next of kin) according to national laws. The patient or 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 septic shock exists and no conscious patients have the combination of severe infection and shock as septic patients have.
No biological material will be collected for the trial, thus no bio-bank will be formed.

1.6 Trial conduct

The trial will adhere to the trial protocol, the Helsinki Declaration in its latest form, good clinical practice (GCP) guidelines and the national laws in the Nordic countries. Inclusion will start after approval by the ethical committees, medicines agencies, data protection agencies and health authorities in the countries of trial sites and trial registration at www.clinicaltrials.gov and European Union Drug Regulating Authorities Clinical Trials (EudraCT).

2. Trial objectives and purpose

The objective is to assess the feasibility of a trial comparing two approaches to fluid resuscitation of septic shock after initial fluid resuscitation; a trigger guided approach vs. a

target guided approach, the latter reflecting standard care. The hypothesis is that the trigger guided approach will result in less fluid given.

3. Trial design

3.1 Trial design

Investigator-initiated multicentre, parallel group, centrally randomised, open label feasibility trial of trigger guided vs. target guided fluid resuscitation in patients with septic shock with adequate generation of allocation sequence, and adequate allocation concealment. Patients will be stratified by centre.

3.2 Randomisation

Randomisation will be centralised, web-based, 24-hour around the clock randomisation according to the allocation list, stratification variable and varying block size created by Copenhagen Trial Unit (CTU) and kept secret for the investigators to allow immediate and concealed allocation to intervention arm. Each patient will be allocated a unique patient ID-number.

3.3 Blinding

Blinding of health care providers will not be feasible which infers that all clinical staff caring for the patients will be aware of the allocation during the intervention period. The two interventions may lead to different use of concomitant interventions, but the lack of blinding may also result in differences in use of concomitant interventions during the intervention period. Hence, we will provide suggestions for use of the relevant co-interventions (see 6.4 concomitant interventions) and will record the use of these.

Information on the primary outcome and other secondary outcomes will be provided by the local investigators from patient charts, but the statistician doing the final analysis will be blinded to which intervention the patients received. Information on whether the exploratory outcome of death occurs will be acquired through public registers (the National Civil Registries) without knowledge of which intervention group the patient was allocated to. The statistician performing analyses will be blinded to allocation and will not receive data on fluid volumes until all other analyses have been performed.

3.4 Participant timeline

We will strive to enrol participants as soon as they fulfil the inclusion criteria in the ICU. Participants will be allocated to a fluid strategy from enrolment until death or ICU discharge. Patients previously enrolled in the CLASSIC trial and readmitted to a trial ICU within the 90 day observation period will re-enter the trial protocol.

4. Selection of participants

All patients referred to a participating clinical trial site will be considered for participation. Patients will be eligible, if they comply with the inclusion and exclusion criteria below.

4.1 Inclusion criteria

- Adult intensive care patients (age ≥ 18 years)

AND

- Sepsis defined as at least 2 of 4 SIRS criteria fulfilled within 24 hours according to Society of Critical Care Medicine/American College of Chest Physicians (SCCM/ACCP):

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. Use the most deranged value recorded* ***in the 24 hours before randomisation***.
2. ***HEART RATE ≥90 beats/minute***. *If patient has 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. ***MECHANICAL VENTILATION*** *for an acute process or respiratory rate ≥ 20 breaths per minute or a $PaCO_2$ < 4.3 kPa (32 mmHg). 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***. *Use the most deranged value recorded* ***in the 24 hours before randomisation***.

AND

- Suspected or confirmed site of infection OR positive blood culture

AND

- Suspected or confirmed circulatory impairment (hypotension/hypoperfusion/hypovolemia) for no more than 12 hours including the hours preceding ICU admission. Circulatory impairment defined as at least one of the following: Systolic blood pressure < 90 mmHg, heart rate > 140 beats/min, lactate ≥ 4 mmol/l, OR use of vasopressors.

AND

- At least 30 ml/kg IBW fluid (colloids, crystalloids or blood products) given in the last 6 hours

AND

- Shock defined as ongoing infusion of noradrenaline (any dose) to maintain blood pressure

4.2 Exclusion criteria

Exclusion criteria:

1. Use of any form of RRT or RRT deemed imminent by ICU physician, i.e. RRT will start within 6 hours
2. Severe hyperkalemia (p-K > 6 mM) within the last 6 hours.
3. Plasma creatinine > 350 µmol/l.
4. Invasively ventilated with FiO_2 > 0.80 and PEEP > 10 cmH_2O
5. Life-threatening bleeding defined as 1) the presence of haemorrhagic shock, as judged by research or treating clinicians or 2) the need for surgical procedure, incl. endoscopy to maintain haemoglobin level.
6. Kidney or liver transplant during current admission.
7. Burns > 10% BSA
8. Previously enrolled in the CLASSIC trial and has finished the 90 day observation period.
9. Patients for whom it has been decided not to give full life support including mechanical ventilation and RRT
10. Consent not obtainable.

4.3 Participant discontinuation and withdrawal

Patients who are withdrawn from the trial protocol will be followed up and analysed as the remaining patients.

4.3.1 Discontinuation and withdrawal at the choice of the participant

The person who has given consent can withdraw his/her consent at any time without need of further explanation, and this will not have any consequences for the participant's further treatment. In order to conduct intention-to-treat analyses with as little missing data as possible, it is in the interest of the trial, to collect as much data from each participant as possible. Therefore, if possible, the investigator will ask the person who has asked for withdrawal which aspects of the trial, s/he wishes the participant to withdraw from:

- receiving the trial intervention;
- participation in the remaining follow-up assessments;
- use of already collected data in the data analyses.

4.3.2 Discontinuation and withdrawal at the choice of the investigator

The investigators may withdraw a participant from the trial intervention at any time at the discretion of treating clinicians.

Patients who will be transferred to another ICU will be withdrawn from the protocol if this ICU is not an active CLASSIC trial site.

4.3.3 Suspension criteria

The protocol may temporarily be suspended for the individual patient, at the discretion of the treating clinicians, if the patient is to be resuscitated with crystalloids in presence of life-

threatening bleeding defined as the presence of haemorrhagic shock, as judged by the investigator or treating clinicians.
The protocol will be resumed promptly once the patient no longer fulfils the suspension criterion. Suspension will not be considered a breach of protocol, and collection of data will continue during the suspension. These patients will be analysed according to their originally assigned groups on an intention-to-treat basis.

5. Selection and trial sites and personnel

5.1 Trial sites and setting

All trial sites will be ICUs. Specific trial sites are listed in the section 'Administrative information'.

5.2 Trial personnel

All clinicians caring for patients in participating ICUs will be eligible to perform the interventions.
All participating ICUs will receive written and oral instructions in how to perform the interventions.

6. Trial interventions

In both intervention arms a target MAP 65 mmHg using noradrenaline is recommended during the entire ICU stay. The target MAP 65 mmHg and choice of noradrenaline as first line vasopressor are based on the SSC 2012 guidelines[6]. A MAP > 65 mmHg in selected patients (e.g. in chronic treatment for arterial hypertension[15]) may be pertinent at the discretion of treating clinicians. Administration of noradrenaline and fluid administration according to the allocated intervention arm may occur concurrently. Figure 2 shows intervention algorithm.

The trial interventions are described in detail as recommended by CONSORT statement for non-pharmacologic trials[16].

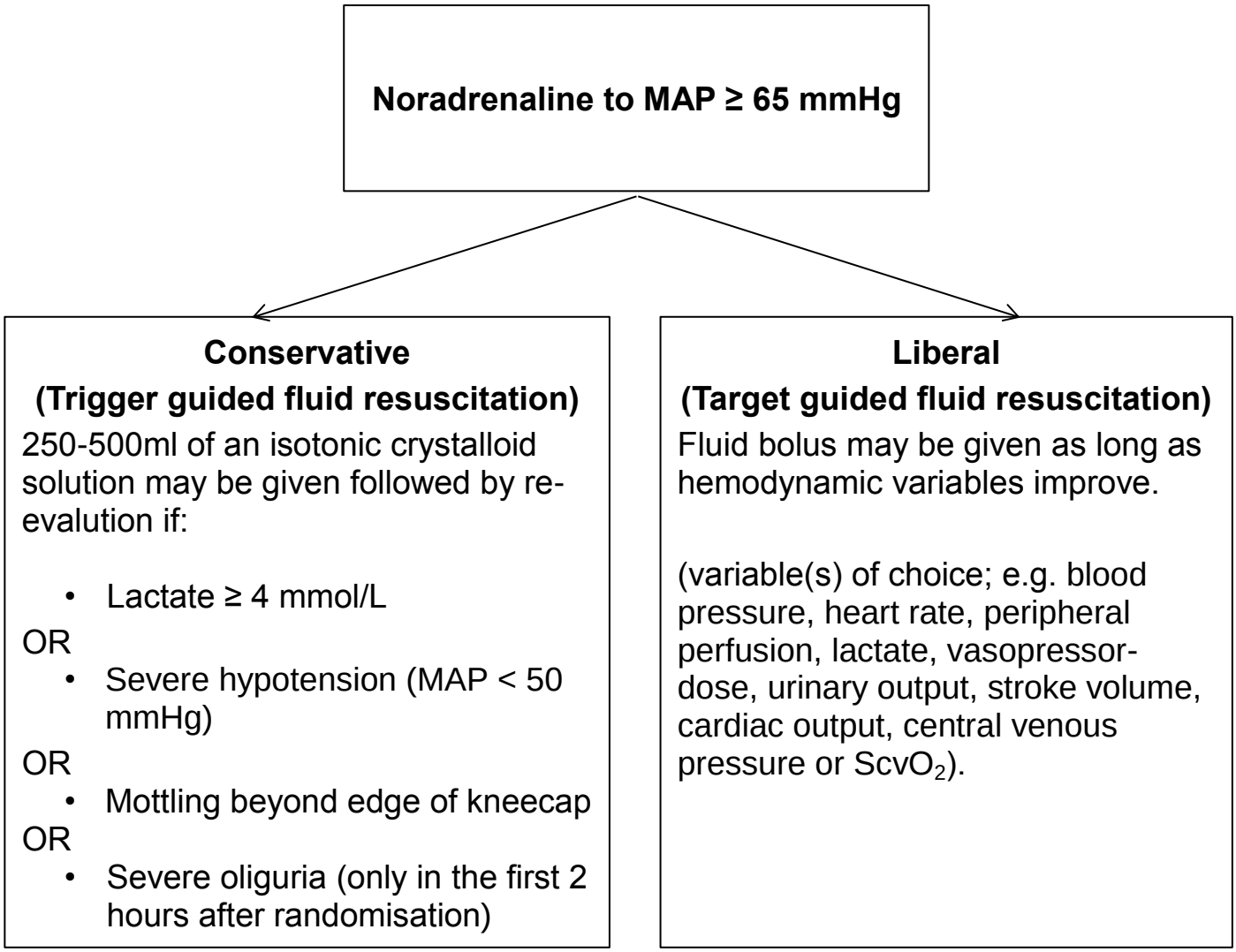

Figure 01. Intervention algorithm.

6.1 Experimental intervention

The experimental intervention is conservative (trigger guided) fluid resuscitation. The intervention period is entire ICU stay.
Trigger guided fluid resuscitation:
A fluid bolus of 250-500 ml may be given if one of the following occurs:

- Plasma lactate concentration ≥ 4mM at point-of-care testing

OR

- Severe hypotension (MAP < 50mmHg)

OR

- Mottling beyond edge of kneecap

OR

- Severe oliguria (only in the first 2 hours after randomisation)

These triggers are chosen because they are markers of hypoperfusion.
A fluid bolus is to be followed by evaluation of effect 30 minutes after the intervention at the latest. If deemed pertinent by the clinicians, further fluid boluses may be given (provided that at least one of the trigger criteria is still fulfilled). Only isotonic crystalloids are to be given as resuscitation fluid; the type of isotonic crystalloid is free of choice. Crystalloid boluses are to be given via IV drip without the use of pressure bags.
The cut-off value of lactate was chosen based on SSC guidelines[6] and data indicating that the marked increase in mortality occur at lactate values above 4 mM[17].
The mottling trigger is based on mottling score ≥ 3 as described by Ait-Oufella et al[18].
Severe oliguria defined as urine output ≤ 0.1 ml/kg/hour IBW last hour.

6.2 Control intervention

The control intervention is liberal (target guided) fluid resuscitation. As for the experimental intervention the intervention period is the entire ICU stay.

Target guided fluid resuscitation:
Fluid boluses may be given as long as hemodynamic variables improve (dynamic or static variable(s) of choice). A fluid bolus is to be followed by evaluation of effect 30 minutes after the intervention at the latest.
'Variable(s) of choice' refers to the variable(s) used to assess hemodynamic improvement.
Fluid resuscitation in this intervention arm is based on SSC 2012, in which the recommendation states: "We recommend that a fluid challenge technique be applied wherein fluid administration is continued as long as there is hemodynamic improvement either based on dynamic (e.g., change in pulse pressure, stroke volume variation) or static (e.g., arterial pressure, heart rate) variables." This recommendation is "ungraded", i.e. not evidence based. We believe this to reflect current practice; that is that clinicians administer fluid as long as they think it improves hemodynamics (the target, which in clinical practice may be any of the

following: blood pressure, heart rate, peripheral perfusion, lactate, vasopressor-dose, urinary output, CVP or SV/CO/ScvO2 etc.).
As in the experimental intervention, only isotonic crystalloids are to be given as resuscitation fluid.

6.3 Co-interventions

See Trial interventions.

6.4 Concomitant interventions

Treatment of septic shock is complex with multiple interventions[6] and as blinding of treating personnel is not feasible use of several concomitant interventions may be influenced by allocated intervention arm. In order to minimise these potential differences suggestions for treatment of following interventions will be provided based on SSC guidelines:

- Vasopressors
- Inotropic agents
- Glucocorticoids
- Blood products
- Renal replacement therapy
- Interventions against atrial fibrillation

Overt fluid losses (e.g. bleeding, diarrhea, ascites and pulmonary effusion) may be substituted in both intervention arms.
Electrolyte and water supplements should be managed enterally if possible.
If total fluid input (including fluid with medication and nutrition) is below 1500 ml/day and water supplements cannot be given enterally, the deficit may be replenished (preferably with intravenous isotonic glucose).

7. Outcome measures

7.1 Primary outcome:

1. Amount of resuscitation fluid given in the first 5 days after randomisation

7.2 Secondary outcomes:

2.1 Amount of resuscitation fluid given during ICU stay
2.2 Fluid balance and total fluid input at day 5 after randomisation and during ICU stay
2.3 Number of patients with major protocol violations
2.4 Accumulated serious adverse reactions (SARs) in ICU.

7.3 Exploratory outcomes:

a. All-cause mortality at day 90

b. Mortality during the total observation time
c. Days alive without use of mechanical ventilation in the 90 days from randomisation.
d. Days alive without use of renal replacement therapy in the 90 days from randomisation.
e. Worsening of acute kidney injury according to KDIGO criteria during ICU stay
f. Delta-creatinine (defined as highest p-creatinine during ICU stay minus most recent p-creatinine prior to randomisation)
g. Ischaemic events in ICU.

Ischaemic events are defined as:
1) Acute myocardial infarction defined as *acute myocardial infarction (ST-elevation myocardial infarction and non-ST elevation myocardial infarction) or unstable angina pectoris*, according to the criteria in the clinical setting in question (e.g. elevated biomarkers, ischaemic signs on ECG, clinical presence) **AND** the patient receives treatment as a consequence of this (reperfusion strategies (PCI/thrombolysis) or initiation/increased antithrombotic drug treatment).
2) Cerebral ischaemia verified by CT- or MR scan.
3) Intestinal ischaemia verified by endoscopy or open surgery.
4) Acute peripheral limb ischaemia: Clinical signs **AND** use of open/percutaneous vascular intervention, amputation or initiation/increased antithrombotic treatment.

8. Safety

8.1 Definitions

Serious adverse event (SAE): any adverse event that results in death, is life-threatening, requires hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability or incapacity

Serious adverse reaction (SAR): any adverse reaction that results in death, is life-threatening, requires hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability or incapacity.

Suspected unexpected serious adverse reaction (SUSAR): any suspected adverse reaction which is both serious and unexpected. SUSARs will be defined as serious reactions not described in the Summaries of Product Characteristics for normal saline, Ringer-lactate, Ringer-acetate and noradrenaline.

8.2 Risk and safety issues in the current trial

Septic shock is characterised by generalised vasodilatation, capillary leaks resulting in impaired perfusion [1,2]. Too conservative fluid resuscitation may potentially result in impaired peripheral and/or organ perfusion. Too liberal fluid resuscitation may potentially result in worsening of tissue edema (including lungs) and lengthen the duration of mechanical ventilation. Liberal fluid resuscitation may also result in increased hemodilution which may induce use of concomitant interventions (e.g. transfusion of blood products).

8.3 Serious adverse reactions and events

The intervention arms in the present trial do not dictate which type of isotonic crystalloid that is to be used. SARs are defined from the Danish summaries of product characteristics of the most frequently used isotonic crystalloids in Denmark (normal saline, Ringer-lactate and Ringer-acetate). Participants in both intervention arms are expected to receive fluid resuscitation, but the amount is hypothesised to differ. Analogously SARs for noradrenaline are defined from the Danish summary of product characteristics. In *appendix 1* all adverse reactions for normal saline, Ringer-lactate, Ringer-acetate and noradrenaline that will not be registered are discussed.

Serious adverse reactions to isotonic crystalloids:

1. Central pontine myolinosis seen on CT or MRI scan.
2. Hyperchloremic acidosis (defined as pH < 7.35 AND p-Chloride > 110 mM)
3. Anaphylactic/allergic reactions
4. Seizures
5. Hypernatremia (defined as p-Na >155 mM)

Serious adverse reactions to noradrenaline:

1. Cerebral haemorrhage seen on CT or MRI scan
2. Cardiac arrhythmia resulting in use of medication or electrical cardioversion
3. Psychiatric symptoms resulting in use of antipsychotic drugs (e.g. haloperidol, quetiapine, risperidone or olanzapine)

Serious adverse events (SAEs) will not be recorded as an entity, because the majority of septic ICU patients will experience several SAEs during their critical illness. The most important SAEs will be captured in the exploratory outcome measures and in the daily SOFA-scoring. Patient charts will contain daily registrations of clinical data, which can be obtained on request from the medical authorities.

Trial investigators are to report SUSARs without undue delay to the sponsor, which in turn will report these to the Danish Health and Medicine Authorities 7 days at the latest after the report has been received.

8.3.1 Recording

Serious adverse reactions (SARs) and suspected unexpected adverse reactions (SUSARs) will be recorded daily in the eCRF during the intervention period.

9. Procedures, assessments and data collection

9.1 Inclusion procedure

9.1.1 Screening

All patients admitted to participating ICUs will be eligible for screening.

9.1.2 Procedures for informed consent

Patients will be included after informed consent according to national laws.
In Denmark patients will be enrolled after informed consent from two physicians, who are independent of the trial (trial guardians). As soon as possible after enrolment, consent will be obtained from the patient's next of kin and general practitioner or the Regional Medical Officer of Health according to Danish law. Patients, who regain consciousness, will be asked for informed consent as soon as possible. The process leading to the achievement of informed consent will be in compliance with all applicable regulations. The consenting party will be provided with written and oral information about the trial so he/she is able to make an informed decision about participation in the trial. The information will be given in a separate room, and the consenting party has the right to bring a companion.
Handling of lack of informed consent in Denmark is described in detail in *appendix 2*.
In Finland: TBD
Written information and the consent form will be subjected to review and approval by the national ethics committee.

9.1.3 Deviation from the standard informed consent in Denmark

According to the standard informed consent form from the National Ethics Committee regarding competent patients, the patient can choose not to receive information regarding new information about the patient's health that arises from the research project. However, the purpose of this project is not to generate new knowledge about the specific patient, so we find that this question is redundant and have omitted the question from the consent form to spare the patient from making unnecessary decisions.

9.2 Data collection

9.2.1 Method

Data will be obtained in eCRFs from a combination of national registers and medical journals. For patients transferred from a trial ICU to non-trial ICU only data obtainable from national registers will be collected after transferral. A bedside CLASSIC resuscitation chart with timing, indication for fluid bolus, fluid type and volume and time to re-evaluation will be recorded and entered in the eCRF.

9.2.2 Timing

Baseline variables

- National identification number
- Sex
- Age at randomisation
- Estimated height and weight
- Co-morbidities (previously admitted for heart failure, myocardial infarction, stroke or asthma or chronic obstructive pulmonary disease Y/N, chronic treatment for arterial hypertension or diabetes Y/N, haematological malignancy Y/N, metastatic cancer Y/N, AIDS 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 habitual s-creatinine.

24-hours prior to randomisation:

- Values for simplified acute physiology score (SAPS II)
- Volume of resuscitation fluids (crystalloids, colloids and blood products specified in ml)
- Results of blood samples (standard lab. values) for haemoglobin (lowest value), lactate (highest value)
- Variables for SOFA scoring *(appendix 3)* not covered above: Lowest mean arterial blood pressure value and highest infusion rate of vasoactive drugs

At randomisation (in the 2 hours prior):

- Highest heart rate
- Lowest values of mean arterial blood pressure, central venous pressure, central venous oxygen saturation obtained from ICU charts
- Highest and lowest values of arterial or venous lactate concentration obtained from ICU charts
- Lowest urinary output (full hour)
- Highest infusion rate of vasoactive drugs

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 ICU charts
- Lowest urinary output (full hour)
- Highest infusion rate of vasoactive drugs

Daily in the first 5 days after randomisation:

- Variables for daily SOFA scoring

- Highest and lowest values of central venous pressure, central venous oxygen saturation and arterial or venous lactate concentration obtained from ICU charts

During the entire ICU stay:

- Daily volumes of resuscitation fluid with specification of indication for each fluid bolus
- Other fluids including blood products, nutrition, fluid with medication, urinary output and calculated fluid balance as of the ICU charts
- Daily lowest values of blood haemoglobin and highest value of plasma lactate and s-creatinine (standard lab. value)
- On mechanical invasive- or non-invasive ventilation Y/N
- Use of renal replacement therapy Y/N
- Use of vasopressors/inotropic agents Y/N
- Use of glucocorticosteroids Y/N
- Interventions against atrial fibrillation
- Ischaemic events
- SARs
- SUSARs

10. Data handling and record keeping

10.1 Data management

Data will be entered into an electronically, web-based eCRF from patient notes by trial personnel. Where appropriate range checks for data entry values will be implemented in the eCRFs.

10.2 Confidentiality

Each patient will receive a unique trial identification number. Trial investigators will receive personal username and passwords to access the CLASSIC trial web-page. Each site will only have access to site specific data.
Data will be handled according to the National Data Protection Agency, and is protected by the Danish national laws “Loven om behandling af personoplysninger” and “Sundhedsloven”.

10.3 Biobanking

No biobank will be formed.

10.4 Access to data

All original records (incl. consent forms, eCRFs, and relevant correspondences) will be archived at trial sites for 15 years. The clean electronic trial database file will be delivered to

the Danish Data Archive and maintained for 15 years and anonymised if requested by the authorities.

11. Statistical plan and data analysis

11.1 Sample size and power

The trial is planned to enrol 150 patients.

11.1.1 Power estimations

Primary outcome:
The primary outcome is amount of resuscitation fluid given at day 5 after randomisation. The power estimation is based on amount of resuscitation fluid given first 5 days in the 6S trial[19].
With 150 included patients we will be able to show a 1.7 L difference in fluid volumes between the groups based on the mean volume of resuscitation fluid given within first 5 days observed in the 6S trial of 5.3 L (SD 3.7 L) with a maximal type 1 and 2 error of 5% and 20% (power=80%), respectively.

Secondary outcomes: All power estimations are based on 150 included patients and a maximal type 1 and 2 error of 5% and 20% (power=80%), respectively.

Resuscitation fluid input during ICU stay: Power to show a 3.7 L difference based on mean of 8.0 L (SD 8.1 L) total fluid input during ICU stay days in the 6S trial.

Total fluid input first 5 days: Power to show a 3.9 L difference based on mean of 17.1 L (SD 8.4 L) total fluid input first 5 days in the 6S trial.

Total input during ICU stay: Power to show a 19.7 L difference based on mean of 39.3 L (SD 42.8 L) total fluid input during ICU stay in the 6S trial.

Cumulative fluid balance on day 5: Power to show a 2.5 L difference based on mean of 6.0 L (SD 5.4 L) cumulative fluid balance on day 5 in the 6S trial.

Cumulative fluid balance during ICU stay: Power to show a 3.7 L difference based on mean of 6.6 L (SD 8.0 L) cumulative fluid balance during ICU stay in the 6S trial.

11.2 Statistical methods

The primary analyses will be an intention-to-treat analysis comparing the two groups by linear regression analysis for continuous outcome measures adjusted for stratification variable (site. An unadjusted Student's t test (or Mann-Whitney if appropriate) for differences in the continuous outcomes will be done as a co-primary analysis. We will perform per protocol analyses of the primary outcome and the secondary outcomes excluding patients with one or more of the following major protocol violation.

1. Fluid bolus given on other indication than specified by the protocol (trigger guided intervention arm)

An analysis comparing indications for resuscitation fluids for the first 30 patients and for the last 30 patients in the target-guided intervention arm will be performed to investigate whether or not a learning effect has occurred during the trial.

11.2.1 Significance
See 11.1

11.2.2 Interim analysis
As this trial is a feasibility trial we do not consider an interim analysis appropriate.

11.2.3 Early stopping criteria
As an interim analysis will not be performed, early stopping criteria will not be applied.

11.2.4 Accountability procedure for missing data/population for analysis
If missing data is > 5% multiple imputations will be performed.

12. Quality control and quality assurance
The coordinating investigator will be responsible for preparing the trial sites including education of local investigators, research nurses, and other trial site personnel before the initiation of the trial. This education will be continuously documented and two annual investigator meetings will be planned.
After initiation, trial site investigators will be responsible for all trial-related procedures at their site, including education of staff in trial-related procedures, recruitment and follow-up of patients and entry of data. Clinical staff at the trial sites will be responsible for the treatment of trial patients.

12.1 Monitoring of the intervention groups
The trial will be externally monitored (the GCP unit at University of Copenhagen) to GCP standards. A centralised day-to-day monitoring of the eCRF will be done by the coordinating investigator.

13. Legal and organisational aspects
13.1 Finance
13.1.1 Trial funding
The CLASSIC trial is third trial of three in the study programme “New Strategies for resuscitation of patients with severe sepsis” and is primarily funded by the Danish Research Council (record number 09-066938). The study programme was granted DKR 14.471.666.

Expectedly DKR 2.100.000 will be allocated for the Classic trial. Additional funding will be applied for. When additional funding is being granted, amount and grant giver will be notified to the Ethics committee and will be added to the informed consent forms.

13.1.2 Compensation

Trial sites will be given DKR 2.000 in case money for each randomised patient to compensate for increased workload participation infers.

13.2 Insurance

In Denmark, all trial participants are insured by the Patient Insurance Association.

13.3 Plan for publication, authorship and dissemination

13.3.1 Publication and authorship

All trial results whether positive, negative or neutral will end up in the public domain, preferably a peer-reviewed publication. All CLASSIC trial sites with patient inclusions will be granted one authorship. From Rigshospitalet and Copenhagen Trial Unit (CTU) as part of the management committee Peter Buhl Hjortrup, Jørn Wetterslev (CTU), Nicolai Haase and Anders Perner will be granted authorship. A trial statistician will also be granted authorship.
The order of authorships will be as follows:
Hjortrup PB, Wetterslev J, Haase N, CLASSIC trial site investigators according to number of included patients, trial statistician and Perner A.

13.4 Spin-off projects (if any)

Presently no spin-off projects are planned.

13.5 Intellectual property rights

Sponsor and primary investigator are prof. Anders Perner. Therefore no contract on intellectual property rights is indicated. The initiative for the Classic trial has been taken by prof. Anders Perner and by doctors at multiple intensive care units, all with no affiliation to institutions that may have economic interests in the trial results.

13.6 Organisational framework

To be finally determined.

13.7 Trial timeline

Tentative trial time line:

April 2014:	Trial sites determined, governance approval applications submitted
June 2014:	First participant enrolled
March 2015:	Last participant enrolled
June 2015:	Follow up completed
October 2015:	Data analysis and submission for publication

14 Appendices

14.1 ***Appendix 1.*** *Adverse reactions*

Normal saline (NaCl):
Hypervolemia in itself is not regarded as a serious adverse reaction, but the potentially serious consequence is reflected in the outcome measure days alive without mechanical ventilation and in the daily SOFA-scoring.

Ringer-lactate:
Sodium retention is not registered as it is not regarded as a serious adverse reaction.
Hyperchloremia is not registered, but is reflected in the SAR hyperchloremic acidosis.

Ringer-acetate:
Heart failure is not directly registered, but is reflected in the outcome measure days alive without mechanical ventilation and in the daily SOFA-scoring.
Conjunctivitis is not registered as it is not regarded as a serious adverse reaction.
Pulmonary edema is not directly registered, but is reflected in the outcome measure days alive without mechanical ventilation and in the daily SOFA-scoring.
Rhinitis is not registered as it is not regarded as a serious adverse reaction.
Overhydration is not directly registered, but is reflected in the outcome measure days alive without mechanical ventilation and in the daily SOFA-scoring.

Noradrenaline:
Change in glucose metabolism is not registered as it is not regarded as a serious adverse reaction.
Tremor, headache, dizziness and sweats are not registered as they are not regarded as serious adverse reactions.
Hypertension is not registered as it is not regarded as a serious adverse reaction.
Dyspnea in itself is not regarded as a serious adverse reaction, but the potentially serious consequence is reflected in the outcome measure days alive without mechanical ventilation and in the daily SOFA-scoring.
Hypersalivation, nausea and vomiting are not registered as they are not regarded as serious adverse reactions.
Urinary retention and difficulty urinating are not registered as they are not regarded as serious adverse reactions.

14.3 ***Appendix 2.*** *Lack of informed consent*

Lack of informed consent from the general practitioner
If the general practitioner cannot be reached (due to vacation, illness or any of the kind) or the patient does not have an assigned general practioner, the Regional Medical Officer of Health will be contacted.

Lack of informed consent from the patient's next of kin
If it is not possible (i.e. contact cannot be obtained) - after obtaining informed consent from two independent physicians and from the patient's general practitioner/the Regional Medical Officer of Health - to obtain informed consent from the patient's next of kin, the patient will continue in the trial until informed consent can be obtained from the patient him-/herself.

Lack of informed consent from the patient's next of kin and the patient deceases
If it is not possible (i.e. contact cannot be obtained) - after obtaining informed consent from two independent physicians and from the patient's general practitioner/the Regional Medical Officer of Health - to obtain informed consent from the patient's next of kin, the patient will continue in the trial until informed consent can be obtained from the patient him-/herself. If the patient deceases before informed consent is obtained, or remains in a permanent state of incompetence, the collected data will be kept and trial outcomes will be obtained centrally.

14.1 Appendix 3. 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	< 400	< 300 (without respiratory support*)	< 200 (with respiratory support*)	< 100 (with respiratory support*)	
(in kPa)	≥53	< 53	< 40 (without respiratory support*)	< 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 is not available, use the value of the latest obtained sample.
*Respiratory support is defined as any form of invasive or non-invasive ventilation including mask CPAP or CPAP delivered through a tracheotomy.

15. References

1. Landry DW, Oliver JA. The pathogenesis of vasodilatory shock. N Engl J Med 2001;345:588–95.

2. Ishihara H, Matsui A, Muraoka M, Tanabe T, Tsubo T, Matsuki A. Detection of capillary protein leakage by indocyanine green and glucose dilutions in septic patients. Crit Care Med [Internet] 2000 [cited 2013 Nov 19];28(3):620–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10752804

3. Karlsson S, Ruokonen E, Varpula T, Ala-Kokko TI, Pettilä V. Long-term outcome and quality-adjusted life years after severe sepsis. Crit Care Med [Internet] 2009 [cited 2013 Nov 19];37(4):1268–74. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19242321

4. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit Care Med [Internet] 2001;29(7):1303–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11445675

5. Linde-Zwirble WT, Angus DC. Severe sepsis epidemiology: sampling, selection, and society. Crit Care [Internet] 2004 [cited 2013 Nov 22];8(4):222–6. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=522859&tool=pmcentrez&rendertype=abstract

6. Dellinger RP, Levy MM, Rhodes A, et al. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012. Crit Care Med 2013;41:580–637.

7. Rivers E, Nguyen B, Havstad S, et al. Early goal-directed therapy in the treatment of severe sepsis and septic shock. N Engl J Med [Internet] 2001 [cited 2013 Nov 8];345(19):1368–77. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11794169

8. Investigators TP. A Randomized Trial of Protocol-Based Care for Early Septic Shock. N Engl J Med [Internet] 2014 [cited 2014 Mar 18];:140318003032001. Available from: http://www.nejm.org/doi/abs/10.1056/NEJMoa1401602

9. Wiedemann HP, Wheeler AP, Bernard GR, et al. Comparison of two fluid-management strategies in acute lung injury. N Engl J Med [Internet] 2006 [cited 2013 Nov 13];354(24):2564–75. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16714767

10. Maitland K, Kiguli S, Opoka RO, et al. Mortality after fluid bolus in African children with severe infection. N Engl J Med [Internet] 2011 [cited 2013 Aug 12];364(26):2483–95. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21615299

11. Boyd JH, Forbes J, Nakada T, Walley KR, Russell J a. Fluid resuscitation in septic shock: a positive fluid balance and elevated central venous pressure are associated with increased mortality. Crit Care Med [Internet] 2011 [cited 2013 Jul 31];39(2):259–65. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20975548

12. Payen D, de Pont AC, Sakr Y, Spies C, Reinhart K, Vincent JL. A positive fluid balance is associated with a worse outcome in patients with acute renal failure. Crit Care [Internet] 2008 [cited 2013 Aug 2];12(3):R74. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2481469&tool=pmcentrez&rendertype=abstract

13. Murphy C V, Schramm GE, Doherty JA, et al. The importance of fluid management in acute lung injury secondary to septic shock. Chest [Internet] 2009 [cited 2013 Nov 19];136(1):102–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19318675

14. Smith SH, Perner A. Higher vs. lower fluid volume for septic shock: clinical characteristics and outcome in unselected patients in a prospective, multicenter cohort. Crit Care [Internet] 2012 [cited 2013 Aug 2];16(3):R76. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3580618&tool=pmcentrez&rendertype=abstract

15. Asfar P, Meziani F, Hamel J-F, et al. High versus Low Blood-Pressure Target in Patients with Septic Shock. N Engl J Med [Internet] 2014 [cited 2014 Mar 18];:140318003032001. Available from: http://www.nejm.org/doi/abs/10.1056/NEJMoa1312173

16. Boutron I, Moher D, Altman DG, Schulz KF, Ravaud P. Annals of Internal Medicine Academia and Clinic Extending the CONSORT Statement to Randomized Trials of Nonpharmacologic Treatment : Explanation and Elaboration. 2008;:295–310.

17. Wacharasint P, Nakada T, Boyd JH, Russell J a, Walley KR. Normal-range blood lactate concentration in septic shock is prognostic and predictive. Shock [Internet] 2012 [cited 2013 Aug 17];38(1):4–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22552014

18. Ait-Oufella H, Lemoinne S, Boelle PY, et al. Mottling score predicts survival in septic shock. Intensive Care Med [Internet] 2011 [cited 2013 Aug 22];37(5):801–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21373821

19. Perner A, Haase N, Guttormsen AB, et al. Hydroxyethyl starch 130/0.42 versus Ringer's acetate in severe sepsis. N Engl J Med [Internet] 2012 [cited 2013 Oct 20];367(2):124–34. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22738085

Statistical Analysis Plan for the Classic Trial

Outcome measures

The outcome measure “Amount of resuscitation fluid given during ICU stay” has been changed from a secondary outcome measure to a co-primary outcome measure, which differs from the Classic Trial protocol. The Classic Trial intervention period is entire ICU stay and we consider it appropriate to have an outcome measure addressing this as co-primary outcome measure. Multiplicity issues will be addressed (see Analyses section).

Co-primary outcome measures:

1.1. Amount of resuscitation fluid given in the first 5 days after randomisation

1.2. Amount of resuscitation fluid given during ICU stay

Secondary outcome measures:

2.1a Fluid balance at day 5 after randomisation

2.1b Fluid balance for entire ICU stay

2.2a Total fluid input at day 5 after randomisation

2.2b Total fluid input during ICU stay

2.3 Number of patients with major protocol violations (violations/length of ICU stay).

Major protocol violation defined as: One or more resuscitation fluid boluses given without fulfilment of one or more of the Classic-criteria in the conservative (Trigger-guided) group.

2.4 Accumulated serious adverse reactions (SARs) in ICU (SARs/length of ICU stay).

Exploratory outcome measures:

a. Death within 90 days following randomisation, Y/N

b.Time to death with censoring on the date at 90 days after the last patient had been randomized. c. Days alive without use of mechanical ventilation (rate: 1-(days with event/days alive(1-90))

d. Days alive without use of RRT (rate: 1-(days with event/days alive (1-90))

e. Worsening of acute kidney injury according to KDIGO criteria during ICU stay as compared to baseline value, Y/N. During the monitoring of missing values it was noted that 10% of the habitual p-creatinine (pre-admission creatinine) values were missing. In these cases the MDRD equation was used to calculate these values. During the monitoring of missing values it was noted that in 2% of the cases the p-creatinine value prior to randomisation was missing and additionally 2% did not have any p-creatinine measurements during their ICU stay. Because of the low number of missing data for worsening of KDIGO score (4%) this will be ignored in that a complete case analysis will be done.

f. Delta-creatinine (defined as highest p-creatinine during ICU stay minus most recent p-creatinine prior to randomisation). During the monitoring of missing values it was noted that in 2% of the cases the p-creatinine value prior to randomisation was missing and additionally 2% did not have any p-creatinine measurements during their ICU stay. Because of the low number of missing delta-creatinine values (4%) this will be ignored in that a complete case analysis will be done.

g. One or more ischemic events in ICU Yes/No.

Analyses

All statistical tests will be 2-tailed.

Multiplicity adjustment. Dealing with multiplicity, the parallel gate keeping method with truncation parameter lambda = 0 will be used to adjust the observed (raw) P values for primary and secondary outcomes (Dmitrienko A, Tamhane AC, Bretz F. Multiple testing problems in pharmaceutical statistics. Chapman & Hall/CRC biostatistics series (2010)).

By this approach the null hypotheses are divided into two families: F1 including null hypotheses related to the two co-primary outcomes and F2 including null hypotheses related to the secondary outcomes. The raw P values are then adjusted. If at least one of the adjusted P values in family 1 is less than the chosen level of significance the hypotheses in family 2 are also tested. If not the hypotheses in family 2 are all accepted without test. However, in all events all raw P values as well as the adjusted ones will be presented.

Lambda may be varied between 0 and 1. If the effect sizes of the primary outcomes (corresponding to the null hypotheses of F1) are uniformly high a lambda near 1 will help improve the overall power. On the other hand if the effect sizes are expected to vary across the endpoints, the overall power is likely to be maximized when lambda is small (Dmitrienko A, Tamhane AC, Bretz F. Multiple testing problems in pharmaceutical statistics. Chapman & Hall/CRC biostatistics series (2010)).

We expect a degree of correlation between the two co-primary outcome measures somewhat in between full correlation and no correlation, so a conventional adjustment of the significance level (0.05/2=0.025) may result in a too conservative adjustment. Thus, we have chosen to adjust the level of significance by a factor in between a full Bonferroni adjustment and no adjustment at all, that is 0.05/1.5=0.033. In the above procedure the raw P values and not the significance level are adjusted and usually α (the significance level) is chosen to be 0.05. In family 1 the smaller raw P value is adjusted by multiplying it with 2. Therefore, we implement the above adjustment solving 2*0.0.033 ≤ level of significance => level of significance = 0.066 to secure that a raw P value ≤0.033 for a co-primary outcome will imply that the corresponding null hypothesis will be rejected.

Revised power calculation
The multiplicity adjustments for the co-primary outcome measures infer changes in the power calculations. The revised power calculations are based on 150 included patients with α=0.033 and β=0.80:
Outcome measure 1.1: Power to show a 1.8 L (opposed to 1.7 L with α=0.05) difference in fluid volumes between the groups based on the mean volume of resuscitation fluid given within first 5 days observed in the 6S trial of 5.3 L (SD 3.7 L)
Outcome measure 1.2: Power to show a 4.1 L (opposed to 3.7 L with α=0.05) difference based on mean of 8.0 L (SD 8.1 L) total resuscitation fluid volume during ICU stay days in the 6S trial.
We regard the revised power to be sufficient to address the research question; thus, the sample size will not be changed.

1. Analysis of outcome measures

Two analyses will be done for the co-primary outcome measures:
(1) an analysis adjusted by the stratification variable (site) - primary analysis
(2) an analysis adjusted by the stratification variable and baseline covariates ((a) surgery during current hospitalisation but prior to randomisation Y/N, (b) Age, (c) more than 5 L of fluid (crystalloids, colloids and blood products combined) given in the 24 hours prior to randomisation Y/N, (d) highest dose of noradrenalin in the 24 hours prior to randomization, (e) estimated weight at randomisation
For exploratory outcome (b) we will perform both an unadjusted analysis (for log rank test) and an analysis adjusted by the stratification variable site.
The remaining outcome measures will only be analysed adjusted by the stratification variable (site).

Co-primary outcome, secondary outcomes 2.1, 2.2 and and exploratory outcome (f) will be analyzed using the general linear model.

The exploratory outcomes (a) and (e) will be analyzed using logistic regression.

The exploratory outcome (b) will be analyzed using Kaplan Meier survival plots and the log rank test. Adjusted analysis will be done using Cox regression model stratified by site.

Secondary outcome 2.3 will not be compared between intervention groups , because major protocol violations can only occur in the conservative (Trigger-guided) group.

Secondary outcomes, 2.4 and exploratory outcomes (c), (d) and (g) will be analyzed using the Poisson distribution with link = log and offset or the negative binomial distribution with link=log and offset as appropriate. As a sensitivity analysis the two groups will also be compared using a non-parametric test (van Elteren test adjusted for site) and major differences in the results obtained by the two approaches will be discussed.

2. **Sensitivity analyses**

The primary outcomes will be analyzed using each of the two per-protocol populations.

Populations

Intention-to-treat population: All randomised patients except those who withdraw their consent for the use of data.

Per-protocol population:

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

1. One or more resuscitation fluid boluses given without fulfilment of one or more of the Classic-criteria in the Conservative (Trigger-guided) group.
   OR
2. Use of colloids (either Albumin or synthetic colloids) for resuscitation
   OR
3. Monitoring revealed that one or more in- or exclusion criteria were violated
   OR
4. Stopped/withdrawn patients

Subgroups:

1. Patients with more than 5 L of fluid (crystalloids, colloids and blood products combined) given in the 24 hours prior to randomisation

The results of the subgroup analysis will be presented if P of test of interaction between subgroup indicator and intervention group indicator for primary outcome is < 0.05. The P-value of the test of interaction will be presented regardless.

Missing Data

Missing primary outcome data:
We do not expect missing data on the co-primary outcome measures. Only complete case analysis will be made.

Missing secondary outcome data
We do not expect missing data on the secondary outcome measures 2.3 and 2.4. Only complete case analysis will be made.
Missing data on secondary outcomes 2.1 and 2.2: Since the predictors (centre indicator and intervention indicator) will not be missing only the outcome may be missing. In this case a complete case analysis will be unbiased since the cases with outcome missing carry no information. However, auxiliary variables (i.e. variables not included in the analytical model such as e.g. other outcomes) may be correlated with the outcome and their inclusion in the analysis will improve the efficiency. This possibility is best dealt with using a structural equation model for the regression analysis with direct maximum likelihood estimation and inclusion of the auxiliary variables (the SAS proc calis for continuous dependent variable may be used). However, the data may still be missing not at random. Therefore, a sensitivity analysis estimating the range of potential bias that may be caused by data missing not at random is done where the missing values in one group are replaced by the minimum value in the whole material and the missing values in the other group are replaced by the maximum value in the whole material and vice versa. The corresponding P values will be estimated. The standard error of each of the two estimates of the regression coefficient will be replaced by the corresponding standard error from the complete case analysis (or the direct ML analysis if auxiliary variables are used) if it is smaller than the former

Missing baseline data

Fluids given prior to randomization Yes/no
The previous approach for multiple imputation we feel had power problems and was too complicated. The simplest and safe approach is to conduct a monotone logistic multiple imputation of the missing baseline covariate. We have therefore changed the approach accordingly (see below)

Some patients may have missing data on fluids given prior to randomisation.
In this case it is a regression of each of the co-primary outcomes on center, and the above mentioned baseline covariates of which only fluids given prior to randomization Yes/no has missing values. Therefore, a multiple imputation of the missing baseline variable will be done using monotone logistic regression.

PAPER III

Effects of fluid restriction on measures of circulatory efficacy in adults with septic shock

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Conflict of interests[1]

[1] The Department of Intensive Care, Rigshospitalet receives research funds from CSL Behring, Switzerland, Fresenius Kabi, Germany, and Ferring Pharmaceuticals, Denmark.

Abstract

Background

The hemodynamic consequences of fluid resuscitation in septic shock have not been fully elucidated. Therefore, we assessed circulatory effects in the first 24 hours of restriction of resuscitation fluid as compared to standard care in intensive care unit (ICU) patients with septic shock.

Methods

This was a post-hoc analysis of the multicentre CLASSIC randomised trial in which patients with septic shock, who had received the initial fluid resuscitation, were randomised to a protocol restricting resuscitation fluid or a standard care protocol in 9 ICUs. The highest plasma lactate, highest dose of noradrenaline, and the urinary output were recorded in five time frames in the first 24 hours after randomisation. We used multiple linear mixed effects models to compare the two groups.

Results

We included all 151 randomised patients; the cumulated fluid resuscitation volume in the first 24 hours after randomisation was median 500 ml (IQR 0-1,500) and 1,250 ml (500-2,500) in the fluid restriction group and standard care group, respectively. The estimated differences in the fluid restriction group vs. the standard care group were 0.1 mM (95% confidence interval -0.7 to 0.9; p=0.86) for lactate, 0.01 µg/kg/min (-0.02 to 0.05; p=0.48) for dose of noradrenaline, and -0.1 ml/kg/h (-0.3 to 0.2; p=0.70) for urinary output during the first 24 hours after randomisation.

Conclusions

We observed no indications of worsening of measures of circulatory efficacy in the first 24 hours of restriction of resuscitation fluid as compared with standard care in adults with septic shock who had received initial resuscitation.

Introduction

The hemodynamic consequences of septic shock are complex and may include hypovolemia due to fluid loss or decreased fluid intake, reduced venous return due to vasodilatation[1], and/or right and/or left ventricular failure.[2] Fluid resuscitation is recommended initially in septic shock and continued fluid administration is suggested as long as the circulation improves[3], which may be evaluated within an hour.[4] However, there are limited outcome data supporting these recommendations[3], and the effects of fluid on markers of circulatory failure is less described, in particular beyond the initial response.

Several parameters may be used as indications for fluid administration in patients with septic shock. Hypotension, vasopressor dosing, and low urinary output were frequent indications for fluid administration in a single centre observational study in intensive care patients with severe sepsis and septic shock.[5] Similarly, hypotension, low urinary output, and high lactate were the most frequently used indications in a global inception cohort study where 27% of the patients had sepsis.[6] Of note, whether the initial response to a fluid bolus was perceived to be positive or negative did not appear to predict further fluid administration.[6]

Septic shock may pose a particular challenge when assessing hemodynamic effects following fluid administration. The increased vascular permeability in septic shock[7] may lead to fast distribution of the infused fluids to the extravascular compartment and thereby shortening the duration of the possible beneficial hemodynamic effects of a fluid bolus. Given these complexities and the paucity of data from randomised clinical trials, we primarily assessed the effects at the group level of being randomised to restricting fluid resuscitation vs. standard care in patients with septic shock who had received initial resuscitation on changes in plasma concentrations of lactate, doses of noradrenaline and urinary outputs during the first 24 hours after randomisation. Overall, we hypothesised that being randomised to a protocol aiming at fluid restriction did not result in worsening in measures of circulatory efficacy in the first 24 hours after randomisation as compared with a protocol aiming at standard care.

Methods

This was a post hoc analysis of the Conservative vs. Liberal Approach to fluid therapy of Septic Shock in Intensive Care (CLASSIC) randomised clinical trial where patients with septic shock who had received the initial fluid resuscitation were randomised to either a protocol restricting fluid resuscitation or a protocol aiming at standard care.[8] Patients were randomised in 9 intensive care units (ICUs) in Denmark and Finland between September 2014 and August 2015. In the fluid restriction group, fluid boluses could be administered – but was not mandated – in case of severe hypoperfusion defined as either plasma lactate above 4 mM, mean arterial pressure (MAP) below 50 mmHg, mottling beyond edge of kneecap, or severe oliguria, the latter only first two hours after randomisation. In the standard care group, fluid boluses could be administered as long as the circulation improved based on variables of the clinicians' choice. The inclusion criteria were: (1) adult in the ICU, (2) fulfilled the criteria for sepsis within the previous 24 hours[9], (3) suspected or confirmed severe circulatory impairment for no more than 12 hours including the hours preceding ICU admission, (4) had received at least 30 mL/kg ideal body weight (IBW) of fluid in the last 6 hours, and (5) had shock defined as ongoing infusion of noradrenaline to maintain blood pressure. The exclusion criteria included any form of renal replacement therapy (RRT), severe hyperkalaemia (p-K > 6 mmol/l) within the last 6 hours, plasma creatinine > 350 μmol/l, mechanical ventilation with FiO_2 > 0.80 and PEEP > 10 cmH_2O, life-threatening bleeding, and patients for whom it had been decided not to give full life support. The full list of exclusion criteria is shown in the Appendix S1. Approvals and written consent from patients and/or legal substitutes were obtained according to national laws. The protocol and the main outcomes have been published.[8] The analyses in this post hoc analysis were defined prior to accessing the variables in question in the database, but after the results of the CLASSIC trial were known. This manuscript was prepared according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement.[10]

Collection of data

The data for the present study were extracted from the CLASSIC trial database. For all fluid resuscitation boluses administered, the amount, timing and type of fluid were recorded. The highest plasma lactate measurements, highest doses of noradrenaline, and the cumulated urinary outputs were recorded in five time frames in the first 24 hours after randomisation (0 to 3 hours, 3 to 6 hours, 6 to 9 hours, 9 to 12 hours and 12 to 24 hours). The noradrenaline doses were recorded in weight adjusted doses. The average hourly urinary output per kg in a given time frame was calculated by dividing the cumulated urinary output with the length of the time frame and the estimated weight. Patient baseline characteristics were also recorded, including patient demographics, co-morbidities and disease severity scores.

Statistical analysis

To assess the effects on changes in plasma lactate concentrations, dose of noradrenaline and urinary output, we used mixed effects models; intra-patient dependencies were accommodated by including random slope and intercept for each patient.

The primary analyses of intervention effect on changes in the plasma lactate, urinary outputs, and doses of noradrenaline were analysed using multiple linear regression adjusted for the stratification variable trial site, and baseline lactate and noradrenaline values as fixed effects and random effects as described above. Thus, three separate analyses for each hemodynamic measure (dependent variables) were performed and values for all five time frames were used in the models. The intervention groups (explanatory variable) were initially allowed to interact with time to allow for the possibility that the treatment effects might vary over time.

The secondary analyses assessed the effect of a time frame with fluid bolus given on changes in the plasma lactate, urinary outputs, and doses of noradrenaline in three separate analyses. Fluid resuscitation (Y/N) in a given time frame (t1) was used as an explanatory variable for changes in the hemodynamic variables (dependent variables). In other words, the dependent variable was the value of the hemodynamic variable at t1 minus the value in the subsequent time frame (t2). For these analyses we used multiple linear regression adjusted for the stratification variable trial site, intervention group, and baseline lactate and noradrenaline values as fixed effects and random effects as described above. The analysis of lactate was further adjusted for use of Ringer's lactate (Y/N) for fluid resuscitation. The dependent variable (fluid resuscitation) group was initially allowed to interact with time to allow for the possibility that the treatment effects might vary over time.

As this was a post hoc study, a sample size calculation was not performed. In order to get an indication of the detectable - or refutable - changes in the three chosen outcomes, we performed calculations for each of the three outcome measures from baseline to the first time frame based on a sample size of 151, α=5% and β=20% (power of 80%). The calculations were simplified and did not take the repeated measures nature of the analyses into account; we therefore expected the actual power to be even higher using the mixed-effects model. According to these estimations we would be able to show a 0.9 mM increase in delta-lactate, a 0.05 µg/kg/min increase in delta-noradrenaline dose, and a 0.5 ml/kg/hour decrease in urinary output. We performed all analyses using SAS software, version 9.4 and considered a 2-sided P value of less than 0.05 to indicate statistical significance.

We *a priori* defined two sensitivity analyses. First, a sensitivity analysis excluding patients with baseline plasma lactate not higher than 2 mM in order to assess the effects in patients with septic shock according

to the Sepsis-3 definition.[11] Second, a sensitivity analysis excluding patients with major violations to the fluid protocol in the fluid restriction group. A major protocol violation was defined as fluid resuscitation bolus given without fulfilment of at least one of the four criteria in the fluid restriction group.

Missing values for the dependent variables were assumed to be missing at random, which is automatically handled by the mixed effects models. True discharge and mortality rates were assumed to be the same between the two treatment groups.

Results

We included all 151 randomised patients in this post hoc analysis. A total of 17 patients (10 patients in the fluid restriction group and 7 patients in the standard care group) were not in the ICU for full 24 hours after randomisation. Of those 5 and 4 had died, and 5 and 3 had been discharged in the fluid restriction group and standard care group, respectively. Patient baseline characteristics stratified by intervention group are presented in **Table 1**. The baseline plasma lactate was above 2 mM in 51 (70%) patients in the fluid restriction group and 43 (59%) in the standard care group.

Missing data

Six patients (4%) had missing baseline value of lactate and /or baseline value of noradrenaline dose and were not included in the mixed effects models. Specifically, 5 patients had missing lactate values at baseline (2 in the fluid restriction group and 3 in the standard care group) and one patient in the standard care group had missing baseline noradrenaline dose. The data availability on lactate measurements, doses of noradrenaline and urinary outputs are presented in Table S3 in Appendix S1.

Fluid resuscitation in the first 24 hours after randomisation

Fluid resuscitation was administered to 43 (57%) patients in fluid restriction group vs. 66 (87%) in the standard care group during median 1 (interquartile range (IQR) 0-3) vs. 2 (1-5) episodes; p=0.001). The cumulated fluid resuscitation volume in the first 24 hours after randomisation was median 500 ml (IQR 0-1,500) and 1,250 ml (500-2,500) in the fluid restriction group and standard care group, respectively ($p < 0.001$ for comparison). In the fluid restriction group, 12 of the 75 patients (16%) had one or more violations of the fluid resuscitation protocol with median 1 (IQR 1-2) violations per patient (Figure S1 in Appendix S1).

Intervention effect on measures of circulatory efficacy

Values of plasma lactate concentrations, doses of noradrenaline, and urinary outputs during the first 24 hours after randomisation did not differ with statistical significance between the two intervention groups (**Table 2)**. There were no indications of interactions with time. Estimated means of each hemodynamic variable for the five time frames in the two groups are presented in **Figure 1**.

Effect of fluid resuscitation on measures of circulatory efficacy

Changes in plasma lactate concentrations and urinary outputs from a time frame with vs. without fluid resuscitation episodes to the subsequent time frame did not differ with statistical significance (**Table 3**). For

changes in noradrenaline dose, we observed a statistically significant interaction with time (p=0.04), but there was no remaining effect if this interaction was not included in the model.

Sensitivity analyses

Sensitivity analyses excluding patients with baseline plasma lactate ≤ 2 and analyses excluding patients with major violations to the fluid protocol in the fluid restriction group did not significantly change the results (Tables S1 and S2 in Appendix S1).

Discussion

In this post hoc analysis of data from a randomised clinical trial of volumes of resuscitation fluids in adults with septic shock, we observed no indications that being randomised to a protocol restricting resuscitation fluids resulted in impairment of measures of circulatory efficacy during the first 24 hours after randomisation as compared with a protocol aiming at standard care with point estimates close to zero. In the secondary analyses, we found that a time frame where fluid resuscitation was not given vs. one where fluid was given did not result in worsening of the measures of circulatory efficacy to the subsequent time frame. The results were supported by pre-defined sensitivity analyses. In the secondary analysis, we observed a statistically significant interaction between the effect of fluid administration and time on change in dose of noradrenaline. This suggests that the effect of fluid resuscitation on the change in dose of noradrenaline depended on which time frame the resuscitation fluid was administered. The estimates from individual time frames suggested that the effect of fluid resuscitation might have been larger in the first hours after randomisation. However, with a borderline significant p-value, this could also have been a spurious finding due to multiple testing.

We hypothesised that being randomised to a protocol restricting volumes of resuscitation fluids would not result in worsening of the hemodynamic variables as compared to standard care. Thus, although the point estimates were close to zero, the confidence intervals are of special importance. For plasma lactate, we observed an upper limit of 0.9 mM increase in the fluid restriction group. Such a difference might be clinically relevant, but in a global survey of 3,000 ICU specialists, more than half responded that a decrease in plasma lactate above 1 mM was needed to constitute a physiological response to a fluid bolus.[12] Also, hyperlactatemia is complex in sepsis, and aetiologies other than hypoperfusion for elevated lactate have been suggested.[13] An observational study in sepsis indicated a non-linear association between lactate and mortality with marked increase occurring around 4 mM.[14] Importantly, in our trial, fluid resuscitation was allowed in both intervention groups in case of plasma lactate above 4 mM. Thus, the between group comparison of changes in plasma lactate did not apply for plasma lactate levels above 4 mM. For dose of noradrenaline, we observed an upper 95% CI limit of 0.05 µg/kg/min higher dose in the fluid restriction group, which arguably is a low potential dose difference with limited clinical relevance. For urinary output we, observed an upper 95% CI limit of 0.2 ml/kg/hour lower urinary output in the fluid restriction group. Over a 24 hour period, this difference would add up to only about half of the observed difference in fluid resuscitation volume between the two groups.

To our knowledge, we are the first to investigate hemodynamic effects of fluid resuscitation in adult patients randomised to receive different fluid resuscitation volumes, and the first to assess the effects beyond the first hours. A systematic review on the physiological changes after fluid bolus therapy in sepsis

published in 2014 found only one observational study assessing hemodynamic effects of fluid resuscitation beyond 60 minutes[15]; this study compared different solutions of hydroxyethyl starches[16] which since has been shown to have detrimental effects in sepsis.[17] Patients with an initial increase in stroke volume and/or cardiac index following a fluid bolus - fluid responders - comprise approximately 50% of critically ill patients.[18] An observational study in 20 patients with shock of whom 70% had septic shock found that the initial increase in cardiac index observed after 30 minutes following a fluid bolus was not sustained after 60 minutes and returned to baseline value after 90 minutes even in fluid responders.[19]

Taken together, there is evidence to suggest that the circulatory efficacy of fluid resuscitation in sepsis may not be present after the initial resuscitation, at least not at the group level. There is also data suggesting potential harmful effects. In children with infection and impaired circulation in a resource-limited region, a fluid bolus of either albumin or saline vs. no bolus had an initial improvement of the circulation after one hour[20], but increased mortality after 48 hours.[21] Additionally, a complex protocol aiming at reducing the fluid balance in patients with ARDS increased the number of ventilator free days.[22] Also, observational studies have associated positive fluid balances with adverse outcomes.[23-26]

Our study has limitations. First, our patients were not randomised to fluid resuscitation vs. no fluid resuscitation at any given time points. Thus, both groups received fluids for circulatory impairment, but in different quantities. Second, the primary analyses comparing the two intervention groups exploited the randomised design of the trial, whereas the secondary analyses of fluid resuscitation episodes did not. The secondary analyses are therefore an observational design which is prone to especially confounding by indication. Third, the statistical analyses assumed that there were no true differences in patients who had been discharged and had died within 24 hours of randomisation between the two groups. We observed similar rates in the two groups, but a violation of this assumption might have changed the results. Fourth, it is possible that a larger difference in volume of resuscitation fluid between the two intervention groups than that observed would result in clinically relevant differences in the measures of circulatory efficacy. Fifth, the median volume of fluid administered prior to randomisation was about 4.5 L in both groups; thus, the results may not be generalizable to the initial fluid resuscitation in septic shock. Last, there may have been some baseline imbalances between the two groups which may have affected the results.

The strength of our study includes lower risk of bias due to the randomised design of the CLASSIC trial. Patients were randomised in multiple ICUs in both university and non-university hospitals, which increases the generalisability of the results.

In conclusion, we observed no indications of worsening of plasma lactate levels, doses of noradrenaline, and urinary output during the first 24 hours after randomisation in adults with septic shock randomised to a protocol restricting fluid resuscitation compared to a protocol aiming at standard care. Both the efficacy

and safety of fluid resuscitation, which is one of the most frequent interventions in sepsis, remain to be established.

Acknowledgments

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References

1. Melo J, Peters JI. Low systemic vascular resistance: differential diagnosis and outcome. Crit Care 1999; 3: 71-77.
2. Parker MM, Shelhamer JH, Bacharach SL, Green MV, Natanson C, Frederick TM, Damske BA, Parrillo JE. Profound but reversible myocardial depression in patients with septic shock. Ann Intern Med 1984; 100: 483-90.
3. Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, Sevransky JE, Sprung CL, Douglas IS, Jaeschke R, Osborn TM, Nunnally ME, Townsend SR, Reinhart K, Kleinpell RM, Angus DC, Deutschman CS, MacHado FR, Rubenfeld GD, Webb S, Beale RJ, Vincent JL, Moreno R. Surviving sepsis campaign: International guidelines for management of severe sepsis and septic shock, 2012. Intensive Care Med 2013; 39: 165-228.
4. Vincent J-L, Weil MH. Fluid challenge revisited. Crit Care Med 2006; 34: 1333-7.
5. Bihari S, Baldwin CE, Bersten AD, Ou J, Holt AW, Prakash S, Schnetz E, Fartasch M, van den Heuvel I, Vlasselaers D, Wouters PJ, Milants I, Ellger B, Vanhorebeek I, Van den Berghe G. Post resusicitation fluid boluses in severe sepsis or septic shock: prevalence and efficacy (price study). Crit Care Resusc 2013; 12: 66-70.
6. Cecconi M, Hofer C, Teboul J-L, Pettila V, Wilkman E, Molnar Z, Della Rocca G, Aldecoa C, Artigas A, Jog S, Sander M, Spies C, Lefrant J-Y, De Backer D. Fluid challenges in intensive care: the FENICE study : A global inception cohort study. Intensive Care Med 2015; 41: 1529-37.
7. Weisel RD, Vito L, Dennis RC, Valeri CR, Hechtman HB. Myocardial depression during sepsis. Am J Surg 1977; 133: 512-21.
8. Hjortrup PB, Haase N, Bundgaard H, Thomsen SL, Winding R, Pettilä V, Aaen A, Lodahl D, Berthelsen RE, Christensen H, Madsen MB, Winkel P, Wetterslev J, Perner A. Restricting volumes of resuscitation fluid in adults with septic shock after initial management: the CLASSIC randomised, parallel-group, multicentre feasibility trial. Intensive Care Med 2016; *In Press*.
9. Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, Schein RM, Sibbald WJ. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. Chest 1992; 101: 1644-55.
10. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. J Clin Epidemiol 2008; 61: 344-49.
11. Shankar-Hari M, Phillips GS, Levy ML, Seymour CW, Liu VX, Deutschman CS, Angus DC, Rubenfeld GD, Singer M, Sepsis Definitions Task F. Developing a New Definition and Assessing New Clinical Criteria for Septic Shock: For the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA 2016; 315: 775-87.
12. Glassford NJ, Mårtensson J, Eastwood GM, Jones SL, Tanaka A, Wilkman E, Bailey M, Bellomo R, Arabi Y, Bagshaw SM, Bannard-smith J, Bin D, Dubin A, Duranteau J, Echeverri J, Hoste E, Joannidis M, Kashani K, Kellum J, Kulkarni AP, Landoni G, Candal CL, Matejovic M, Yunos M, Anaes M, Nichol A, Straaten HOV, Perner A, Pettila V, Reinhart K, Richards G, Schneider AG. Defining the characteristics and expectations of fluid bolus therapy: A worldwide perspective. J Crit Care 2016; 35.
13. Jansen TC, van Bommel J, Bakker J. Blood lactate monitoring in critically ill patients: a systematic health technology assessment. Crit Care Med 2009; 37: 2827-39.
14. Wacharasint P, Nakada T-a, Boyd JH, Russell JA, Walley KR. Normal-range blood lactate concentration in septic shock is prognostic and predictive. Shock (Augusta, Ga) 2012; 38: 4-10.
15. Glassford NJ, Eastwood GM, Bellomo R. Physiological changes after fluid bolus therapy in sepsis: a systematic review of contemporary data. Crit Care 2014; 18: 696.

16. van Haren FM, Sleigh J, Boerma EC, La Pine M, Bahr M, Pickkers P, van der Hoeven JG. Hypertonic fluid administration in patients with septic shock: a prospective randomized controlled pilot study. Shock 2012; 37: 268-75.
17. Haase N, Perner A, Hennings LI, Siegemund M, Lauridsen B, Wetterslev M, Wetterslev J. Hydroxyethyl starch 130/0.38-0.45 versus crystalloid or albumin in patients with sepsis: systematic review with meta-analysis and trial sequential analysis. BMJ 2013; 346: f839.
18. Michard F, Teboul JL. Predicting fluid responsiveness in ICU patients: a critical analysis of the evidence. Chest 2002; 121: 2000-8.
19. Nunes TS, Ladeira RT, Bafi AT, de Azevedo LC, Machado FR, Freitas FG. Duration of hemodynamic effects of crystalloids in patients with circulatory shock after initial resuscitation. Ann Intensive Care 2014; 4: 25.
20. Maitland K, George EC, Evans Ja, Kiguli S, Olupot-Olupot P, Akech SO, Opoka RO, Engoru C, Nyeko R, Mtove G, Reyburn H, Brent B, Nteziyaremye J, Mpoya A, Prevatt N, Dambisya CM, Semakula D, Ddungu A, Okuuny V, Wokulira R, Timbwa M, Otii B, Levin M, Crawley J, Babiker AG, Gibb DM. Exploring mechanisms of excess mortality with early fluid resuscitation: insights from the FEAST trial. BMC Med 2013; 11: 68-68.
21. Maitland K, Kiguli S, Opoka RO, Engoru C, Olupot-Olupot P, Akech SO, Nyeko R, Mtove G, Reyburn H, Lang T, Brent B, Evans JA, Tibenderana JK, Crawley J, Russell EC, Levin M, Babiker AG, Gibb DM. Mortality after Fluid Bolus in African Children with Severe Infection. N Engl J Med 2011; 364: 2483-95.
22. Wiedemann HP, Wheeler AP, Bernard GR, Thompson BT, Hayden D, DeBoisblanc B, Connors AF, Hite RD, Harabin AL. Comparison of two fluid-management strategies in acute lung injury. The New England journal of medicine 2006; 354: 2564-75.
23. Boyd JH, Forbes J, Nakada T-a, Walley KR, Russell JA. 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.
24. Acheampong A, Vincent J-L. A positive fluid balance is an independent prognostic factor in patients with sepsis. Crit Care 2015; 19: 251-51.
25. Vincent J-L, Sakr Y, Sprung CL, Ranieri VM, Reinhart K, Gerlach H, Moreno R, Carlet J, Le Gall J-R, Payen D, Investigators SOIAIP. Sepsis in European intensive care units: results of the SOAP study. Crit Care Med 2006; 34: 344-53.
26. Vaara ST, Korhonen A-M, Kaukonen K-M, Nisula S, Inkinen O, Hoppu S, Laurila JJ, Mildh L, Reinikainen M, Lund V, Parviainen I, Pettilä V. Fluid overload is associated with an increased risk for 90-day mortality in critically ill patients with renal replacement therapy: data from the prospective FINNAKI study. Critical Care 2012; 16: R197-R97.

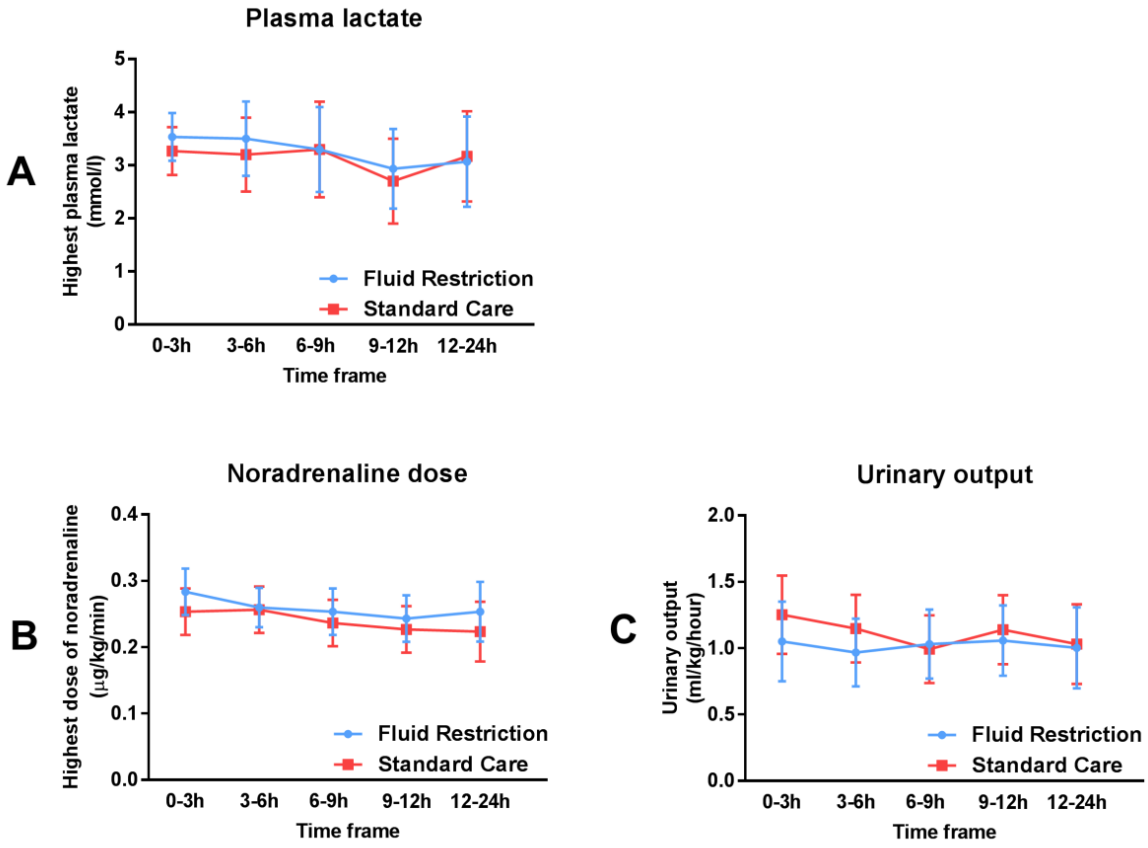


Figure 1. Hemodynamic variables in patients with septic shock in 5 time frames during the first 24 hours after randomisation to fluid restriction or standard care

The graphs depict estimated means with 95% confidence limits (error bars).

Panel A shows the estimated means of highest plasma lactate in each time frame adjusted for trial site and baseline plasma lactate. Panel B shows the estimated for highest dose of noradrenaline in each time frame adjusted for trial site and baseline dose of noradrenaline. Panel C shows the estimated means for hourly urine output in each time frame adjusted for trial site.

Table 1. Patient baseline characteristics

	Fluid restriction group (n=75)		Standard care group (n=76)	
	Fluid bolus first 24 hours (n=43)	**No bolus first 24 hours (n=32)**	**Fluid bolus first 24 hours (n=66)**	**No bolus first 24 hours (n=10)**
Male gender, No. (%)	30 (70)	22 (69)	53 (64)	5 (50)
Age, years	71 (60-76)	68 (62-76)	73 (67-77)	70 (59-81)
Weight, kg	80 (65-90)	77 (62-85)	71 (62-82)	79 (66-90)
Hypertension, No. (%)	17 (40)	18 (56)	27 (41)	2 (20)
Hematological malignancy, No. (%)	4 (9)	1 (3)	7 (11)	1 (10)
Surgery, No. (%)[a]	25 (58)	22 (68)	34 (52)	6 (60)
Source of ICU admittance, No. (%)				
Emergency department	10 (23)	8 (25)	17 (26)	0 (0)
General ward	17 (40)	11 (35)	22 (33)	5 (50)
Operating room or recovery room	14 (33)	13 (40)	24 (36)	5 (50)
Other ICU	2 (4)	0 (0)	3 (5)	0 (0)
Source of sepsis, No. (%)[b]				
Lungs	12 (28)	11 (34)	33 (50)	3 (30)
Abdomen	23 (53)	15 (47)	29 (44)	4 (40)
Urinary tract	5 (12)	3 (9)	10 (15)	0 (0)
Soft tissue	6 (14)	7 (22)	2 (3)	3 (30)
Other	3 (7)	4 (13)	6 (9)	1 (10)
Hours from ICU admission to randomisation	3.5 (1.7-7.3)	6.0 (2.2-10.1)	3.8 (1.6-6.2)	4.8 (1.5-6.4)
SAPS II[c]	57 (44-63)	47 (40-55)	56 (47-66)	56 (44-67)
SOFA score[d]	11 (9-12)	8 (7-10)	10 (9-12)	9 (7-10)
Acute kidney injury, No. (%)[e]	22 (52)	16 (50)	24 (38)	4 (40)
Mechanical ventilation, No. (%)[f]	23 (53)	18 (56)	38 (58)	5 (50)
Highest lactate, mM[g]	3.9 (2.5-6.5)	2.6 (1.6-3.4)	2.6 (1.6-5)	1.5 (1.1-2.2)
Highest dose of noradrenaline, µg/kg/min[h]	0.25 (0.16-0.40)	0.16 (0.10-0.32)	0.20 (0.10-0.30)	0.25 (0.12-0.30)
Highest heart rate, beats/min[g]	113 (97-128)	98 (94-121)	107 (89-125)	112 (85-115))
Highest plasma creatinine, µmol/L[h]	142 (85-197)	128 (79-157)	110 (81-195)	114 (76-159)
Highest plasma sodium, mmol/L[h]	138 (134-140)	140 (137-142)	138 (135-142)	137 (128-140)
Fluid prior to randomisation, mL[h]	4,100 (3,465-6,500)	4,300 (3,197-7,302)	4,440 (3,232-6,510)	7,520 (5,135-9,157)

Values with ranges are medians (interquartile ranges).

Abbreviations: ICU, intensive care unit; SAPS, Simplified Acute Physiology Score; SOFA, Sequential Organ Failure Assessment.

[a] During the hospital admission but prior to randomisation.

[b] Some patients had more than one source of infection.

[c] SAPS II in the 24 hours prior to randomisation. One or 2 of the 17 variables used to calculate the score were missing in 39; their scores were not included here.

[d] SOFA score in the 24 hours prior to randomisation. One or 2 of the 5 sub-scores used to calculate the score were missing in 32 patients; their scores were not included here.

[e] Acute kidney injury defined as the KDIGO Creatinine score > 0 in the 24 hours prior to randomisation. Data were missing for 3 patients; their scores were not included here.

[f] Invasive or non-invasive ventilation in the 24 hours prior to randomisation.

[g] In the 3 hours prior to randomisation.

[h] In the 24 hours prior to randomisation.

Table 2. Between group comparisons of changes in hemodynamic variables during the first 24 hours after randomisation

	Fluid restriction group vs. Standard care (95% CI)	P-value for comparison	P-value for interaction with time
Lactate, mM	0.1 (-0.7 to 0.9)	0.86	0.88
Noradrenaline dose, µg/kg/min	0.01(-0.02 to 0.05)	0.48	0.67
Urinary output, ml/kg/hour	-0.1 (-0.3 to 0.2)	0.70	0.78

Linear mixed effects models adjusted for baseline plasma lactate, baseline dose of noradrenaline, and trial site as fixed effects. Abbreviations: CI, confidence interval.

Table 3. Changes in hemodynamic variables from a time frame with vs. without fluid resuscitation episodes to the subsequent time frame.

	Time frame with fluid resuscitation vs. time frame without fluid resuscitation (95% CI)	P-value	P-value for interaction with time
Lactate, mM	-0.1 (-0.5 to 0.3)	0.59	0.90
Urinary output, ml/kg/hour	0.1 (-0.1 to 0.3)	0.25	0.99
Noradrenaline dose, µg/kg/min	0.01 (-0.01 to 0.04)	0.25	0.04
Comparisons for individual time frames due to significant interaction with time			
0-3 hours	-0.03 (-0.06 to 0.01)		
3-6 hours	-0.01 (-0.05 to 0.04)		
6-9 hours	0.06 (0.02 to 0.11)		
9-12 hours	0.07 (0.02 to 0.12)		

Linear mixed effects models adjusted for baseline plasma lactate, baseline dose of noradrenaline, and trial site as fixed effects. The analysis for plasma lactate was further adjusted for use of Ringer's lactate. Abbreviations: CI, confidence interval.

Appendix S1

Article: Effects of fluid restriction on measures of circulatory efficacy in adults with septic shock: post hoc analyses of a randomised clinical trial

Table of Contents

Table S1. Sensitivity analyses for between group comparisons of changes in hemodynamic variables during the first 24 hours after randomisation

	Fluid restriction group vs. Standard care (95% CI)	P-value for comparison	P-value for interaction with time
Excluding patients with baseline lactate ≤ 2 mmol/l			
Lactate, mM	0.1 (-1.1 to 1.4)	0.82	0.99
Noradrenaline dose, µg/kg/min	0.00 (-0.82 to 0.82)	0.91	0.49
Urinary output, ml/kg/hour	0.1 (-0.3 to 0.4)	0.70	0.13
Excluding patients with major protocol violations			
Lactate, mM	0.0 (-0.9 to 0.9)	0.96	>0.99
Noradrenaline dose, µg/kg/min	-0.01 (-0.04 to 0.03)	0.80	0.83
Urinary output, ml/kg/hour	0.1 (-0.2 to 0.4)	0.56	0.85

Linear mixed effects models adjusted for baseline plasma lactate, baseline dose of noradrenaline, and trial site as fixed effects. Abbreviations: CI, confidence interval.

Table S2. Sensitivity analyses for changes in hemodynamic variables from a time frame with vs. without fluid resuscitation episodes to the subsequent time frame.

	Fluid resuscitation episode vs. no fluid resuscitation episode (95% CI)	P-value for comparison	P-value for interaction with time
Excluding patients with baseline lactate ≤ 2 mmol/l			
Lactate, mM	-0.2 (-0.7 to 0.3)	0.43	0.91
Noradrenaline dose, µg/kg/min	0.01 (-0.02 to 0.05)	0.46	0.23
Urinary output, ml/kg/hour	0.1 (-0.1 to 0.4)	0.37	0.99
Excluding patients with major protocol violations			
Lactate, mM	-0.1 (-0.5 to 0.3)	0.56	0.95
Noradrenaline dose, µg/kg/min	0.01 (-0.02 to 0.04)	0.41	0.27
Urinary output, ml/kg/hour	0.1 (-0.1 to 0.4)	0.29	0.99

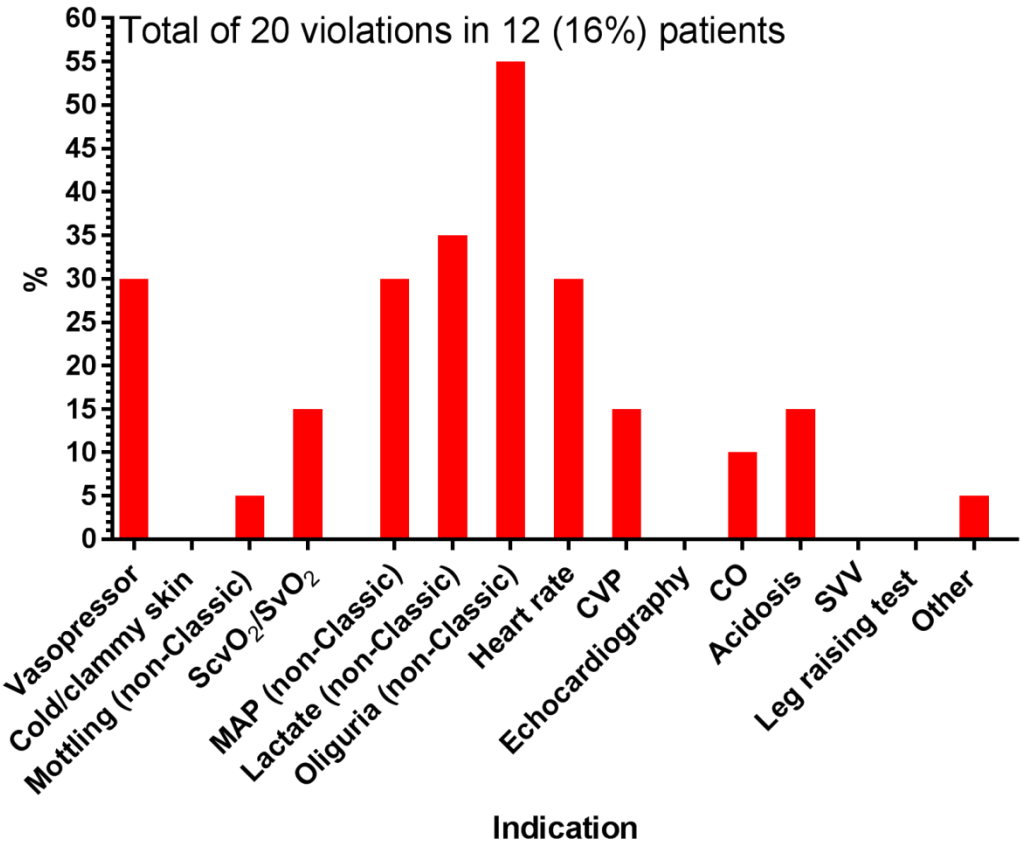
Linear mixed effects models adjusted for baseline plasma lactate, baseline dose of noradrenaline, and trial site as fixed effects. The analysis for plasma lactate was further adjusted for use of Ringer's lactate. Abbreviations: CI, confidence interval.

Table S3. Data availability for each time frame

	0-3 hours	3-6 hours	6-9 hours	9-12 hours	12-hours
Lactate	142 (94)	134 (89)	129 (85)	123 (81)	130 (86)
Noradrenaline	150 (99)	149 (99)	148 (98)	138 (91)	134 (89)
Urinary output	149 (99)	150 (99)	149 (99)	138 (91)	135 (89)

Data are presented as number of patients (%) with available data.

Figure S1. Indications for fluid resuscitation violating the protocol in the fluid restriction group in first 24 hours after randomisation.



CLASSIC trial exclusion criteria

1. Use of any form of renal replacement therapy (RRT) or RRT deemed imminent by ICU physician, i.e. RRT would start within 6 hours
2. Severe hyperkalemia (p-K > 6 mmol/l) within the last 6 hours.
3. Plasma creatinine > 350 µmol/l.
4. Invasively ventilated with FiO_2 > 0.80 and positive end-expiratory pressure (PEEP) > 10 cmH_2O
5. Life-threatening bleeding defined as 1) the presence of haemorrhagic shock, as judged by researcher or treating clinicians or 2) the need for surgical procedure, incl. endoscopy to maintain haemoglobin level.
6. Kidney or liver transplant during present admission.
7. Burns > 10% body surface area
8. Previously enrolled in the CLASSIC trial and had finished the 90 day observation period.
9. Patients for whom it had been decided not to give full life support including mechanical ventilation and RRT
10. Consent not obtainable.