

Levosimendan to prevent acute organ dysfunction in sepsis: the LeoPARDS RCT

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**National Institute for
Health Research**

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Abstract

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Background: In septic shock, cardiovascular resuscitation using catecholamine vasopressors and inotropes is standard therapy, but catecholamines have important side effects. Levosimendan (Simdax®; Orion Pharma, Newbury, UK) is a calcium-sensitising drug with inotropic and other properties that may have a role in sepsis.

Objectives: To determine, in adult septic shock, whether or not levosimendan reduces the incidence and severity of acute organ dysfunction, the effect of levosimendan on individual organ function and the safety profile of levosimendan.

Design: Multicentre, randomised, double-blind, parallel-group, placebo-controlled study.

Setting: UK intensive care units.

Participants: Adult patients with sepsis and cardiovascular failure requiring vasopressors to maintain blood pressure despite adequate fluid resuscitation.

Intervention: Levosimendan, at a dosage of 0.05–0.2 µg/kg/minute, compared with placebo for 24 hours, in addition to standard care, within 24 hours of meeting inclusion criteria.

Main outcome measure: The primary outcome was mean Sequential Organ Failure Assessment (SOFA) score on the intensive care unit after randomisation to a maximum of 28 days. Secondary outcomes were time to extubation, survival up to 6 months and serious adverse events.

Results: In total, 2382 patients were screened at 34 centres, of whom 516 were randomised to treatment, 259 to levosimendan and 257 to placebo. Baseline characteristics were well balanced across treatment arms. There was no significant difference in mean \pm standard deviation (SD) SOFA score between the levosimendan group (6.7, SD 4.0) and the placebo group (6.1, SD 3.9) [mean difference 0.61, 95% confidence interval (CI) -0.07 to 1.29]. The 28-day mortality rate was 34.5% and 30.9% in the levosimendan and placebo groups, respectively (absolute difference 3.6%, 95% CI -4.5% to 11.7%). Patients in the levosimendan group were less likely to be successfully extubated over 28 days than patients in the placebo group (hazard ratio 0.77, 95% CI 0.60 to 0.97). More patients in the levosimendan group had supraventricular tachyarrhythmias (3.1% vs. 0.4%; absolute difference 2.7%, 95% CI 0.1% to 5.3%), but there was no overall difference in serious adverse events.

Conclusions: In the population of septic shock patients randomised to treatment in this study, the addition of levosimendan to standard medical care did not reduce organ dysfunction or mortality. Levosimendan was associated with a reduced likelihood of successful extubation and an increased risk of supraventricular tachyarrhythmias.

Limitations: This was a trial of levosimendan added to standard care rather than a comparison against an alternative inotrope such as dobutamine. No echocardiographic analyses were performed to provide detailed information about changes in myocardial function; therefore, this trial cannot provide guidance as to which inotrope (if any) is best to use in the management of sepsis if a very low cardiac index is present.

Future work: Levosimendan could be compared against dobutamine and placebo in patients with a very low cardiac output in sepsis to test which, if any, inotrope should be used in this select group.

Trial registration: Current Controlled Trials ISRCTN12776039.

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List of supplementary material

Report Supplementary Material 1 Supplementary methods and results

Supplementary material can be found on the NIHR Journals Library report project page (www.journalslibrary.nihr.ac.uk/programmes/eme/111408/#/documentation).

Supplementary material has been provided by the authors to support the report and any files provided at submission will have been seen by peer reviewers, but not extensively reviewed. Any supplementary material provided at a later stage in the process may not have been peer reviewed.

List of abbreviations

AE	adverse event	MAKE	major acute kidney event
AKIN	Acute Kidney Injury Network	MAP	mean arterial pressure
APACHE II	Acute Physiology and Chronic Health Evaluation II	MCMC	Markov chain Monte Carlo
AR	adverse reaction	MHRA	Medicines and Healthcare products Regulatory Agency
ATP	adenosine triphosphate	NIHR	National Institute for Health Research
AUC	area under the concentration–time curve	NT-proBNP	N-terminal prohormone of brain natriuretic peptide
CCL2	chemokine ligand 2	OR	odds ratio
CI	confidence interval	PaO_2	arterial partial pressure of oxygen
C_{max}	maximum concentration	PI	principal investigator
CRF	case report form	REC	Research Ethics Committee
CrI	credible interval	RRT	renal replacement therapy
CRN	Clinical Research Network	SAE	serious adverse event
DMEC	Data Monitoring and Ethics Committee	SaO_2	arterial oxygen saturation
eCRF	electronic case record form	SAP	statistical analysis plan
EDC	electronic data capture	$ScvO_2$	central venous oxygen saturation
EME	Efficacy and Mechanism Evaluation	SD	standard deviation
FI_{O_2}	fraction of inspired oxygen	SIRS	systemic inflammatory response syndrome
GCP	Good Clinical Practice	SmPC	Summary of Product Characteristics
GP	general practitioner	SOFA	Sequential Organ Failure Assessment
HME	heat and moisture exchange	sTNFR1	soluble tumour necrosis factor receptor 1
ICTU	Imperial Clinical Trials Unit	SUSAR	suspected unexpected serious adverse reaction
ICU	intensive care unit	TMF	Trial Master File
IL	interleukin	TMG	Trial Management Group
IMP	investigational medicinal product	TSC	Trial Steering Committee
IQR	interquartile range	VANISH	Vasopressin vs Noradrenaline as Initial therapy in Septic Shock
ITT	intention to treat		
LeoPARDS	Levosimendan for the Prevention of Acute oRgan Dysfunction in Sepsis		
LOCF	last observation carried forward		

Plain English summary

Septic shock is a common life-threatening condition. It occurs when infection causes blood pressure to fall to dangerous levels, reducing blood flow to vital organs. Adrenaline-like drugs are commonly used to improve a patient's blood pressure, but they can have serious side effects. Levosimendan is a new type of drug that works in a different way from adrenaline-like drugs, potentially avoiding these side effects.

The LeoPARDS (Levosimendan for the Prevention of Acute oRgan Dysfunction in Sepsis) trial investigated whether or not using levosimendan to treat patients who had septic shock improved the function of different organs and improved recovery.

Patients who had septic shock were randomised to receive a 24-hour infusion of levosimendan or placebo, in addition to standard care. Patients were followed up daily for up to 28 days and then at 6 months. A total of 2382 patients were assessed and, of these, 516 patients from 34 intensive care units across the UK took part in the trial from January 2014 to December 2015.

Levosimendan did not improve organ function or any other outcomes when added to standard care in septic shock. Levosimendan is known to dilate blood vessels and this might improve the blood supply to various organs. However, in patients receiving levosimendan, higher doses of adrenaline-like drugs had to be given to maintain blood pressure because of the dilatation of the blood vessels. The patients then had a faster heart rate and more irregular heartbeats. In addition, those patients who required help with breathing from a ventilator required this support for longer.

We cannot recommend the use of levosimendan to treat routine cases of septic shock. Although it is disappointing that this study has not shown a benefit from using levosimendan in sepsis, it has still provided useful information. Knowing when not to use drugs is important to reduce the risk of side effects and to avoid wasting precious resources.

Scientific summary

Background

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. Septic shock is the most severe form of sepsis and results in circulatory and metabolic abnormalities and is a leading cause of death worldwide. Sepsis is responsible for approximately 30% of all admissions to intensive care in the UK; despite improvements in care, the mortality rate remains high.

Catecholamines are the recommended first-line therapy for septic shock; however, high doses of administered and circulating catecholamines are associated with poor outcomes and severe side effects, including myocardial injury and peripheral ischaemia. A combination of vascular hyporeactivity to catecholamines, myocardial depression and profound vasodilatation can lead to persisting hypotension despite adequate fluid resuscitation.

Levosimendan (Simdax®; Orion Pharma, Newbury, UK) is a calcium-sensitising drug with inotropic and vasodilatory properties licensed for the treatment of acute heart failure. Levosimendan sensitises the myocardium to calcium through binding to troponin C, so that a greater ventricular contraction and stroke volume can be achieved for the same level of intracellular calcium.

When compared with catecholamine use, levosimendan shows an increased myocardial contraction with a minimal increase in oxygen demand, and diastolic relaxation is not affected. Levosimendan also mediates vasodilatation by opening adenosine triphosphate (ATP)-sensitive potassium channels in vascular smooth muscle and may have cardioprotective effects.

Several small studies have investigated levosimendan in human septic shock and reported an improvement in haemodynamics, microcirculatory flow and renal and hepatic function. A recent meta-analysis supported its use in sepsis; however, only 125 patients in total were treated.

The LeoPARDS (Levosimendan for the Prevention of Acute oRgan Dysfunction in Sepsis) trial was designed to determine whether or not levosimendan, when added to standard care, could reduce organ dysfunction in septic shock and to assess its safety profile in this group of patients.

Methods

Trial design and participants

The LeoPARDS trial was a multicentre, double-blind, placebo-controlled randomised clinical trial conducted in 34 general adult intensive care units (ICUs) in the UK.

The London – Harrow Research and Ethics Committee approved the trial (reference no. 13/LO/0365). Written consent was obtained from either the patient or, in the event of a lack of capacity, a personal or professional legal representative prior to enrolment into the trial. Retrospective written consent was sought from patients once they regained capacity.

Adult patients (aged ≥ 18 years) who had at least two of four systemic inflammatory response syndrome criteria as a result of known or suspected infection, who had received vasopressors for ≥ 4 hours despite adequate intravenous fluid resuscitation and who were deemed to have an ongoing vasopressor requirement were eligible for inclusion. Patients had to be recruited within 24 hours of meeting the inclusion criteria.

Exclusion criteria were as follows:

- > 24 hours elapsed since meeting all of the inclusion criteria
- end-stage renal failure
- chronic severe hepatic impairment
- history of torsades de pointes
- significant mechanical obstruction affecting ventricular filling and/or outflow
- a treatment limitation decision was in place
- body weight of > 135 kg
- pregnancy
- treated with levosimendan within the previous 30 days
- hypersensitivity to levosimendan or any of its excipients
- enrolled in another interventional trial that might interact with the study drug.

Randomisation and masking

Enrolment, randomisation and data collection were performed via an online system. Patients were assigned to levosimendan or placebo on a 1 : 1 basis with variable block size concealed randomisation using computer-generated random numbers and were stratified by recruitment centre.

Vials of levosimendan and the matching placebo were supplied by Orion Corporation Orion Pharmaceuticals (Espoo, Finland). Trial-specific labelling and packaging, to ensure that trial packs were identical, were undertaken by Victoria Pharmaceuticals (Belfast, UK). Patients and clinical and research staff remained blinded to treatment allocation throughout the trial.

Clinical management

Patients received all normal standards of care and, in addition, were allocated to receive a blinded infusion of either levosimendan or placebo for 24 hours. No bolus loading dose was given. The study drug was commenced at a rate of 0.1 µg/kg/minute and, if tolerated, was increased after 2–4 hours to 0.2 µg/kg/minute for a further 20–22 hours. Patients received intravenous fluid bolus(es) for any clinically significant drop in blood pressure and, if necessary, vasopressors were titrated to maintain an adequate blood pressure. If the 0.2 µg/kg/minute dose was not tolerated, because of either hypotension or severe tachycardia, the infusion rate was reduced to 0.1 µg/kg/minute. If still not tolerated, the rate was reduced to 0.05 µg/kg/minute and, if still not tolerated, the treatment was discontinued.

Other aspects of clinical care were at the local physicians' discretion and based on the Surviving Sepsis Campaign guidelines (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. *Crit Care Med* 2013;**41**:580–637). The study protocol recommended crystalloid infusions as the resuscitation fluid of choice, with noradrenaline as the initial vasopressor, but vasopressin or its analogues could be added to maintain a mean arterial pressure (MAP) of 65–70 mmHg. The MAP target could be varied for individual patients, but investigators were encouraged to use the lowest dose of vasopressor to maintain an acceptable MAP that maintained tissue perfusion in each patient. Hydrocortisone could be added for patients who were poorly responsive to vasopressors (i.e. on high doses of vasopressors). Additional inotropic agents could be used in either treatment group, as clinically indicated, that is, for those with ongoing low cardiac output after fluid resuscitation. Dobutamine was the inotropic agent of choice, with down-titration and discontinuation once an adequate oxygen delivery had been achieved.

Outcome measures

The primary outcome measure of the trial was the mean daily Sequential Organ Failure Assessment (SOFA) score while in the ICU from randomisation to a maximum of 28 days. The daily SOFA score was calculated for each patient based on five organ systems: cardiovascular, respiratory, renal, hepatic and coagulation (maximum score 20). The neurological system was not included because of the difficulties of accurately scoring the Glasgow Coma Scale score daily in the presence of sedation. Daily scores were totalled for

each patient's ICU stay and divided by the number of days they remained in the ICU to calculate the mean SOFA score for each patient.

To assess the effect of levosimendan on individual organ systems, as well as to analyse the individual SOFA components, several clinical outcomes were determined a priori for secondary analyses. These included the number of catecholamine- and ventilator-free days, the time to successful extubation, the proportion of patients with a major acute kidney event over 28 days (defined as death, new requirement for renal replacement therapy or sustained renal failure at day 28) and duration of renal replacement therapy. Mortality rates at 28 days, at ICU and hospital discharge, and at 3 and 6 months, as well as ICU length of stay and serious adverse event rates, were also recorded.

Statistical analysis

A sample size of 500 was chosen to provide 90% power to detect a 0.5-point difference in mean SOFA score assuming a standard deviation (SD) of 1.5 and a significance level of 0.05. To allow for a 3% withdrawal of consent, the recruitment target was 516 patients.

The primary analysis was an unadjusted, intention-to-treat analysis and reported the difference in mean SOFA scores between the two treatment groups. As levosimendan is a known inotrope but is not included as part of the cardiovascular scoring within the SOFA score, a sensitivity analysis was carried out by repeating the primary analysis but excluding the cardiovascular component.

Four subgroup analyses were planned a priori based on baseline measurement of the cardiac index, if measured (lowest tertile vs. middle and highest tertiles); central venous saturations (three groups: low < 70%, normal 70–85%, high > 85%); serum lactate (≤ 2 vs. > 2 mmol/l); and noradrenaline (below vs. above the median infusion rate). The heterogeneity of treatment effect according to subgroup was calculated using a permutation test, permuting both the subgroup and the treatment allocation. All analyses were carried out using R version 3.2.2 (The R Foundation for Statistical Computing, Vienna, Austria), with a p -value of < 0.05 considered statistically significant using two-sided tests.

Results

The trial ran from January 2014 until December 2015, when the required sample size was achieved. Seven patients did not receive the allocated study drug. One patient in the placebo group received open-label levosimendan after receiving the blinded study drug. Two patients in each group died before the study drug could be administered. One levosimendan group patient rapidly improved after randomisation and one placebo group patient was randomised during a temporary halt in recruitment and so was not administered the study drug. These seven patients were included in the analysis. The family of one patient in the levosimendan group withdrew consent after randomisation but before the study drug was administered. This patient was excluded from all analyses.

The two groups were well balanced at baseline and typical of a sick group of septic shock patients, with a median Acute Physiology and Chronic Health Evaluation II (APACHE II) score of 25 (interquartile range 21–30) and a median serum lactate level of 2.3 mmol/l (interquartile range 1.4–3.6 mmol/l). The median time to recruitment was 16 hours after starting vasopressors, and the median dose of noradrenaline was 0.28 μ g/kg/minute to achieve a MAP of 74 mmHg (interquartile range 68–79 mmHg) at the time of starting the study drug.

Cardiovascular effects

Thirty-three patients (13.5%) in the levosimendan group stopped the study drug infusion before the 24-hour time point because of haemodynamic instability (hypotension or tachycardia) compared with 19 (7.7%) in the placebo group. The MAP was lower in levosimendan-treated patients in the first 24 hours, but was similar after that time in both groups. The rate and duration of noradrenaline infusion was

higher in the levosimendan group, although there was less frequent use of dobutamine. Heart rate was significantly higher in levosimendan-treated patients over the first 4 days. Intravenous fluid administration, fluid balance and serum lactate levels were similar in both groups.

The primary outcome, the mean SD daily SOFA score over the ICU stay, was 6.7 (SD 4.0) in the levosimendan group and 6.1 (SD 3.9) in the placebo group [mean difference 0.61, 95% confidence interval (CI) -0.07 to 1.29]. After adjusting for ICU, age and APACHE II score in a regression model, the mean difference was 0.59 (95% CI -0.02 to 1.20). When considering each component of the total SOFA score independently, the mean daily cardiovascular score was higher in the levosimendan group than in the placebo group (mean difference 0.25, 95% CI 0.04 to 0.46). As a prespecified analysis, the primary analysis was repeated excluding the cardiovascular component of the SOFA score, giving a mean daily SOFA score of 4.4 SD 3.1 in the levosimendan group and 4.1 (SD 3.1) in the placebo group (mean difference 0.36, 95% CI -0.17 to 0.90).

The mortality rate at 28 days was 34.5% in the levosimendan group and 30.9% in the placebo group (absolute difference 3.6%, 95% CI -4.5% to 11.7%). Patients in the levosimendan group were less likely to be successfully extubated over 28 days than patients in the placebo group (hazard ratio 0.76, 95% CI 0.60 to 0.97). The median number of ventilator-free days was 16 in the levosimendan group and 19 in the placebo group (difference -3.0 days, 95% CI -9.5 to 1.0 days). The number of catecholamine-free days was 22 and 23 in the levosimendan and placebo groups, respectively (difference -1.0 days, 95% CI -4.5 to 1.0 days). In total, 32 levosimendan-treated patients experienced a serious adverse event, compared with 23 patients in the placebo group; supraventricular arrhythmias were more common in the levosimendan group.

No differences in the primary outcome and 28-day mortality rate were seen in any of four predefined subgroup analyses, and there was no significant heterogeneity of treatment effect in any subgroup.

Discussion

In this multicentre, double-blind randomised clinical trial levosimendan did not reduce organ dysfunction when added to standard care for adult patients suffering from septic shock. Patients treated with levosimendan required more noradrenaline, had a higher heart rate and were mechanically ventilated for longer.

Cardiovascular resuscitation is an essential component of sepsis management. However, there is increasing evidence that high doses of catecholamine infusions are associated with worse outcomes. Alternative non-catecholamine vasopressor and inotrope options are thus being investigated. Levosimendan offers an inotropic action through different mechanisms from those of catecholamines. Although levosimendan has a half-life of about 1 hour, its active metabolite, OR-1896, has a long half-life. A single 24-hour infusion should provide haemodynamic effects over a week, long enough to cover the majority of cases of septic shock.

Levosimendan has other important non-inotropic effects. It opens ATP-sensitive potassium channels in vascular smooth muscle, leading to vasodilatation. It may also be protective to the heart and other organs, especially in ischaemia/reperfusion injury. Additional properties include anti-inflammatory, antioxidative and antiapoptotic effects.

In view of these pleiotropic effects and the fact that myocardial dysfunction, although present in > 50% of the septic shock population, may not be clinically evident even when using cardiac output monitoring, we recruited all patients who had septic shock. We also planned four subgroup analyses to examine the effect of levosimendan in higher risk patients, including those with a low cardiac output, those with impaired oxygen delivery to the tissues and those on high doses of catecholamines. There was no evidence of a beneficial effect of levosimendan in any of these prespecified subgroups.

Although levosimendan does not stimulate beta-adrenoreceptors, a significantly higher heart rate was seen in the levosimendan group, most likely as a result of vasodilatation although possibly related to the increased requirement for noradrenaline. Similarly, there was a higher rate of tachyarrhythmias in levosimendan-treated patients, and this may have contributed to the lack of overall clinical benefit.

Patients in the levosimendan group were less likely to be successfully weaned from mechanical ventilation over 28 days. Levosimendan has been reported to sensitise the diaphragmatic muscle to calcium, improve contractility and reverse the development of fatigue after muscle loading. Combined with the prolonged inotropic effect of levosimendan and its active metabolite, levosimendan might have been expected to improve ventilator weaning. It remains unclear why the opposite effect was seen.

There were limitations of the study. This was a trial of levosimendan added to standard care rather than a comparison of levosimendan against an alternative inotrope such as dobutamine. Fewer than 10% of patients in the placebo group received dobutamine. There was no difference in outcome between the groups in the prespecified subgroup analysis of patients with a low cardiac index. In addition, no echocardiographic analyses were performed to provide more detailed information about changes in myocardial function with levosimendan treatment. Therefore, this trial cannot provide guidance as to which inotrope is best to use in the management of sepsis if a very low cardiac index is present.

Conclusions

Among adult patients with septic shock, levosimendan when added to standard care does not reduce organ dysfunction or mortality. Patients allocated to the levosimendan group were less likely to be successfully extubated, had more tachycardia and had a higher rate of supraventricular arrhythmias than those allocated to the placebo group. Therefore, levosimendan cannot be recommended for routine use in septic shock.

Trial registration

This trial is registered as ISRCTN12776039.

Funding

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Chapter 1 Introduction

Background

Severe sepsis is responsible for approximately 30% of all admissions to intensive care in the UK, yet, despite improvements in care, the mortality rate from severe sepsis remains high.¹ According to data from the Intensive Care National Audit & Research Centre, the incidence of severe sepsis has increased by 68% over a 9-year period, such that the total number of severe sepsis cases in the UK is in excess of 45,000 per annum, and the hospital mortality rate is approximately 45%.¹ Mortality rates increase with increasing number of organ failures.^{2,3} In particular, acute renal failure in severe sepsis is an independent risk factor for death [odds ratio (OR) 2.1].⁴

It was estimated in 2001 that treating critically ill patients who have sepsis costs the NHS more than £700M.⁵ As the population ages and receives more complex medical treatments, the incidence of sepsis, and of associated mortality and morbidity, and costs will continue to rise. Therefore, severe sepsis is an extremely important health-care problem.

Levosimendan

Levosimendan (Simdax®; Orion Pharma, Newbury, UK) is a licensed treatment for decompensated heart failure in > 50 countries around the world. It acts by sensitising the myocardium to calcium so that a greater ventricular contraction (and thus stroke volume) can be achieved for the same intracellular calcium concentration, thereby reducing the workload of the failing heart.⁶ The drug itself has a short plasma half-life of approximately 1 hour, is around 95% bound to plasma proteins and is fully metabolised in the liver and intestine into both active and inactive metabolites. However, the haemodynamic effects are maintained for up to 7 days after a single 24-hour infusion of levosimendan because of the effects of the active metabolite, OR-1896, which has an elimination half-life of approximately 80 hours.⁷

Extensive animal and human investigations have concluded that the mechanism of action of levosimendan also includes vasodilatation, mediated by activation of adenosine triphosphate (ATP)-sensitive sarcolemmal potassium channels and ATP-sensitive mitochondrial potassium channels.⁸ This in turn may lead to the maintenance of mitochondrial volume and a reduction in calcium overload seen in ischaemia, thereby preserving mitochondrial function.⁹ Levosimendan has also been shown to possess anti-inflammatory properties.^{10,11} Post licensing, levosimendan has been extensively investigated in patients with acute heart failure caused by a variety of aetiologies.

As part of the systemic inflammatory response, myocardial dysfunction is seen in > 50% of patients with severe sepsis.¹² The likely mechanism of myocardial dysfunction is a combination of altered calcium trafficking and reduced troponin sensitivity to calcium¹³ and its presence contributes to multiple organ failure including acute renal failure. The calcium-sensitising and anti-inflammatory actions of levosimendan provide a strong biological rationale for its use in sepsis. In addition, conventional vasoactive support using catecholamines such as noradrenaline and dobutamine may result in sympathetic overstimulation and a range of adverse effects.¹⁴ Evidence of lack of benefits from trials comparing different catecholamine regimens,¹⁵ increased mortality in patients exposed to a greater vasopressor load¹⁶ and the observation of higher plasma catecholamine levels in non-survivors than in survivors of critical illness¹⁷ all provide further evidence of possible harm from conventional catecholamine therapy.

Levosimendan in sepsis: animal studies

The use of levosimendan in severe sepsis has been studied in a range of animal models of sepsis. In an ovine septic shock model, the combination of levosimendan (0.2 µg/kg/minute) and vasopressin as opposed to noradrenaline and vasopressin was associated with improved myocardial, pulmonary and renal function.¹⁸ In a mouse model of septic shock, Zager *et al.*¹⁹ observed that levosimendan protected against acute renal failure, probably because of vasodilatation in the kidney as a result of levosimendan inducing ATP-sensitive potassium channel activation. Similarly, in a rat model of sepsis, Fries *et al.*²⁰ demonstrated an improvement in microvascular perfusion in the buccal mucosa of animals given 0.3 µg/kg/minute of levosimendan. Dubin *et al.*²¹ evaluated higher doses of levosimendan (100-µg loading dose followed by an infusion of 1.6 µg/kg/minute) in an ovine model, demonstrating that levosimendan prevented the reduction in mesenteric oxygen delivery that was seen in the control group animals. In a porcine septic shock model, high-dose levosimendan attenuated the increase in pulmonary vascular resistance and improved both hepatosplanchnic and systemic blood flow compared with control animals.²² An improved responsiveness to noradrenaline was also seen in the porcine septic shock model study.

In two studies from the same group,^{23,24} both in porcine models of sepsis, levosimendan failed to show an improvement in hepatosplanchnic perfusion compared with both placebo and dobutamine, as had been seen in other studies. These results were felt, in part, to be the result of the failure to adequately fluid resuscitate the animals and restore mean arterial pressure (MAP) prior to commencing levosimendan.²⁵

Levosimendan in sepsis: human studies

In humans, evidence of a potential benefit from levosimendan in severe sepsis comes from a combination of case reports and case series,^{26–29} together with a number of clinical trials.^{30–35} In a case series of six patients with refractory septic shock given 0.1–0.2 µg/kg/minute of levosimendan, there was a trend towards improved haemodynamics, associated with a reduction in catecholamine requirements.²⁹ All but one of these patients survived to hospital discharge despite an Acute Physiology and Chronic Health Evaluation II (APACHE II)-predicted mortality of 60%.²⁹

Three of the sepsis trials in humans have been led by Dr Morelli. The first trial, published in 2005, compared an infusion of 0.2 µg/kg/minute of levosimendan with an infusion of 5 µg/kg/minute of dobutamine for 24 hours in 28 patients with septic shock and echocardiographically proven acute left ventricular dysfunction.³⁰ Statistically significant reductions in both pulmonary artery pressure and pulmonary artery occlusion pressure and an increase in left ventricular stroke work index were seen with levosimendan. In particular, levosimendan increased creatinine clearance by 64% while decreasing serum lactate levels compared with dobutamine.³⁰ In 35 patients with septic shock and the acute respiratory distress syndrome, 0.2 µg/kg/minute of levosimendan increased the cardiac index and reduced the mean pulmonary artery pressure compared with placebo.³¹ More recently, in a study of 40 patients who had septic shock, the effects of levosimendan (0.2 µg/kg/minute) on microcirculatory blood flow in the sublingual mucosa were compared with those of dobutamine (5 µg/kg/minute). Blood flow was significantly higher in the levosimendan group ($p < 0.001$) and there was a trend towards higher central venous oxygen saturation (ScvO₂) and arterial pH as well as lower noradrenaline requirements in the levosimendan group.³² These studies were not of sufficient size to detect any differences in any patient-focused outcomes.

A number of other small trials have been performed. In 42 patients with septic shock, levosimendan compared with dobutamine reduced the number of patients requiring additional catecholamine support with noradrenaline ($p < 0.04$).³³ In a similar trial, 42 patients who had severe sepsis and a cardiac index of < 2.2 l/minute/m² received either levosimendan or dobutamine as additional therapy. Cardiac index, ejection fraction and ScvO₂ all increased significantly more in the levosimendan group than in the dobutamine group.³⁴ In a more recent trial of 30 patients with septic shock, patients randomised to receive levosimendan

(0.1 µg/kg/minute) had significantly improved splanchnic perfusion as measured by the indocyanine green plasma disappearance rate than those randomised to receive dobutamine (10 µg/kg/minute).³⁵

Further evidence of a beneficial effect of levosimendan on renal function in sepsis comes from a case–control study of 99 patients with septic shock who received 0.2 µg/kg/minute of levosimendan for 24 hours within 36 hours of admission to the intensive care unit (ICU). Compared with matched control patients, a 24% increase in glomerular filtration rate at 96 hours ($p < 0.05$) was seen in patients who received levosimendan together with a lower peak serum creatinine concentration ($p < 0.05$).³⁶ Similar beneficial effects of levosimendan on renal function have also been demonstrated in patients with acute heart failure. In 88 patients who had acute decompensated heart failure requiring inotropic therapy, levosimendan compared significantly increased the calculated glomerular filtration rate with dobutamine, with an increase of 45% seen at 72 hours after infusion completion.³⁷

There are no existing systematic reviews of levosimendan in severe sepsis. However, a recent article reviewing the role of levosimendan in sepsis concluded that 'large-scale multicenter clinical trials are now needed to clarify whether levosimendan improves the overall outcome of patients with sepsis and septic shock'.³⁸

Risks and benefits

The potential benefits of levosimendan have been reviewed in the previous sections. Levosimendan has been widely used in patients with acute heart failure, has a good safety profile and has no known significant pharmacokinetic drug interactions. According to the levosimendan investigators' brochure, between September 2000 (when the drug first received a licence in Sweden) and November 2010, an estimated 440,000 patients were treated with levosimendan, with a reported serious adverse drug reaction rate of 791/≈440,000 (0.2%). The most common events reported were hypotension (0.03%) and serious arrhythmias (0.02%).

Levosimendan has been used in > 200 patients with septic shock in published controlled trials and case series without any reported significant adverse effects. Adequate cardiovascular resuscitation with intravenous fluids and noradrenaline, as well as avoiding an initial bolus dose and high-dose infusion (≥ 0.4 µg/kg/minute), help reduce adverse effects when used in sepsis.³⁸

Levosimendan is currently used in many ICUs within Europe in the treatment of severe sepsis and septic shock and has recently been recommended as an alternative inotrope in the German Sepsis Society guidelines.³⁹

As highlighted earlier, septic shock is associated with a high mortality rate and many of the drugs required for its treatment, for example high-dose catecholamine infusions, also have significant risks. Available evidence would suggest that levosimendan has a good safety profile and would not add any additional risk in this population. In fact, levosimendan may reduce the risk associated with standard therapy if catecholamine use is reduced.

Toxicology

Conventional studies on general toxicity and genotoxicity have revealed no special hazard for humans with short-term use of levosimendan. In animal studies, levosimendan was not teratogenic, but it caused a generalised reduction in the degree of ossification in rat and rabbit fetuses, with anomalous development of the supraoccipital bone in the rabbit. Pregnant patients were not included in this trial.

Rationale for study

As summarised earlier, there is a substantial body of research that provides proof of concept that levosimendan improves cardiac output, regional perfusion and other physiological end points, including creatinine clearance and glomerular filtration rate, in patients who have septic shock.

We undertook an exploratory trial, designed to identify the important clinical outcome benefits and to explore the mechanism of action of levosimendan in septic shock. Given that multiple organ dysfunction is associated with an increased mortality rate,⁴⁰ a reduction in the incidence and severity of organ failure would be associated with meaningful benefits to patients and clinicians alike, along with potential reductions in costs to the NHS. The trial protocol⁴¹ and the main outcomes of the trial⁴² have been previously published.

Chapter 2 Research objectives

Objectives

Primary objectives

The main objectives of this trial were to:

- ascertain if levosimendan reduces the incidence and severity of organ dysfunction compared with placebo in adult patients who have septic shock
- identify the effect of levosimendan on individual organ function in septic shock
- establish the safety profile and pharmacokinetics of levosimendan in this group of patients.

Secondary objectives

The secondary objectives were to:

- identify whether or not levosimendan reduces the need for, and duration of, catecholamine support and thus reduces myocardial injury
- establish whether or not levosimendan alters the pro- and anti-inflammatory balance in sepsis
- collect long-term (3- and 6-month) survival data to help inform the appropriate long-term outcome measure for a subsequent effectiveness trial, should the efficacy of levosimendan be confirmed in this trial.

Chapter 3 Methods

Trial design

This was a multicentre, randomised, double-blind, placebo-controlled study. Eligible patients were randomised within 24 hours of meeting all of the inclusion criteria. Patients were randomised to receive either levosimendan or placebo in addition to standard care. The duration of therapy with the study medication (active or placebo) was 24 hours; data were collected daily up to 28 days while in the ICU, with long-term follow-up at 3 and 6 months after hospital admission.

Table 1 summarises the visit and data collection schedule for the study.

Randomisation and blinding

Patients were assigned to either levosimendan or placebo in a 1 : 1 ratio. Randomisation was stratified by ICU in permuted blocks of variable sizes of four and six. The randomisation lists were computer generated by a statistician independent of the LeoPARDS (Levosimendan for the Prevention of Acute oRgan Dysfunction in Sepsis) trial team and held separately at the Imperial Clinical Trials Unit (ICTU). The randomisation lists were imported into the InForm system (version 4.6; Oracle Corporation, Red Wood City, CA, USA) to enable web-based randomisation at sites. Study drugs were labelled and packaged identically by Victoria Pharmaceuticals (Belfast, UK) to preserve blinding. Patients, clinical staff and the research team were blinded to drug allocation with the exception of trial statisticians Greg Frazier, Alexina Mason and Shalini Santhakumaran. The senior statistician, Deborah Ashby, remained blinded throughout the trial.

TABLE 1 Visit and data collection schedule

Visit	Day							
	1	2	3	4	5	6	7	8–28
Screening	✓							
Informed consent (patient consent/PerLR/ProLR/retrospective patient information and consent)	Patient/PerLR/ProLR will be obtained initially. Retrospective patient consent will be obtained when the patient has recovered							
Inclusion/exclusion criteria	✓							
Randomisation	✓							
Study drug administration	Study drug infusion for 24 hours							
Follow-up								
Blood and urine sampling	✓	✓	✓	✓	✓	✓		Patients in the pharmacokinetic study had additional blood sampling at days 8, 10, 13 and 16
Daily collection of clinical data	✓	✓	✓	✓	✓	✓	✓	✓
Final visit	On the day of discharge from hospital							

PerLR, personal legal representative; ProLR, professional legal representative.

Participants

Participants in the trial were adult patients who had sepsis and cardiovascular failure requiring vasopressors to maintain blood pressure despite adequate fluid resuscitation.

Inclusion criteria

Inclusion criteria were the internationally established consensus definitions of sepsis. In brief:

- Fulfil two out of four of the criteria of the systemic inflammatory response syndrome (SIRS) because of known or suspected infection within the previous 24 hours. The SIRS criteria are:
 - fever ($> 38\text{ }^{\circ}\text{C}$) or hypothermia ($< 36\text{ }^{\circ}\text{C}$)
 - tachycardia (heart rate > 90 beats per minute)
 - tachypnoea (respiratory rate > 20 breaths per minute or $\text{PaCO}_2 < 4.3\text{ kPa}$) or need for mechanical ventilation
 - abnormal leucocyte count [$> 12,000\text{ cells/mm}^3$, $< 4000\text{ cells/mm}^3$ or $> 10\%$ immature (band) forms].
- Hypotension, despite adequate intravenous fluid resuscitation, requiring treatment with a vasopressor infusion (e.g. noradrenaline/adrenaline/vasopressin analogue) for ≥ 4 hours and still having an ongoing vasopressor requirement at the time of randomisation.

Exclusion criteria

- More than 24 hours since meeting all of the inclusion criteria.
- End-stage renal failure at presentation (previously dialysis dependent).
- Severe chronic hepatic impairment (Child–Pugh class C).
- A history of torsades de pointes.
- Known significant mechanical obstructions affecting ventricular filling or outflow or both.
- Treatment limitation decision in place [e.g. DNAR (do not attempt resuscitation) or not for ventilation/dialysis].
- Known or estimated weight of $> 135\text{ kg}$.
- Known to be pregnant.
- Previous treatment with levosimendan within 30 days.
- Known hypersensitivity to levosimendan or any of the excipients.
- Known to have received another investigational medicinal product (IMP) within 30 days or currently in another interventional trial that might interact with the study drug.

Treatments

Patients were randomised to either the treatment group or the control group.

The study drug was not started until the treating physician was confident that adequate fluid resuscitation had been achieved and the patient had reached the target MAP (suggested target 65–70 mmHg but this could be varied, as detailed below). Adequate fluid resuscitation was achieved using repeated fluid challenges. Examples of appropriate targets included any or all of the following:

- central venous pressure $\geq 8\text{ mmHg}$ ($\geq 12\text{ mmHg}$ in mechanically ventilated patients)
- good peripheral perfusion on clinical examination
- other measures of cardiac output/flow (e.g. stroke volume variability, global end-diastolic volume index).

Treatment group

Patients in the treatment group received all normal standard care plus a 24-hour blinded intravenous infusion of levosimendan. The levosimendan infusion started at 0.1 µg/kg/minute and, if tolerated, this was increased after 2–4 hours to 0.2 µg/kg/minute for a further 20–22 hours (total infusion of 24 hours). Levosimendan can cause vasodilatation. Therefore, it was advised that if there was a mild drop in blood pressure an intravenous fluid bolus should be given (e.g. 250–500 ml), fluid status should be reassessed and treatment given as necessary. The vasopressor dose could then be titrated up if needed once any fluid depletion had been corrected. The infusion rate would then be increased after 2–4 hours once the clinician was satisfied that the drug was well tolerated.

If the dose of 0.2 µg/kg/minute was not tolerated (hypotension despite titration of vasopressors, or severe tachycardia), the rate of infusion was reduced back to 0.1 µg/kg/minute. If there was hypotension or tachycardia at an infusion rate of 0.1 µg/kg/minute (either initially or later) then the rate of infusion was reduced to 0.05 µg/kg/minute. If the hypotension or tachycardia continued then the infusion was discontinued (see *Figure 1*).

The Summary of Product Characteristics (SmPC) states that an initial bolus of levosimendan should be given followed by a 24-hour infusion of 0.1–0.2 µg/kg/minute (reduced if there is hypotension or tachycardia) when treating acute decompensated heart failure. To avoid hypotension and to maintain safety in this septic shock population during the LeOPARDS trial a bolus dose was never given. The infusion was started at the lower dose to ensure that the drug was well tolerated by patients before being increased to the higher dose. The dose of 0.2 µg/kg/minute has shown clinical benefit in previous septic shock clinical trials and, importantly, has been shown to be safe using detailed global haemodynamic and microcirculatory monitoring.^{30–32} The titration of the dose between 0.05 and 0.2 µg/kg/minute helped ensure that patients received an effective dose but that any adverse effects were minimised in each individual patient.

Control group

Patients in the control group received all normal standard care plus a 24-hour blinded intravenous infusion of matching placebo. The placebo infusion rate followed the treatment group regimen.

During the study drug administration period, and especially during the first 6 hours, patients were repeatedly reassessed to ensure adequate fluid resuscitation using any or all of the targets above.

The study drugs were supplied to the ICU by the local pharmacy as specific research study drugs and were stored in separate research stores (e.g. locked boxes/fridges in the ICU). The study drug was drawn up and administered by the bedside critical care nurse (or critical care research nurses). The study drug was prescribed on the patient drug chart by the clinical staff as per the policy of each ICU. Preprinted stickers or preset electronic prescriptions were provided to ensure standardised prescribing, dilution and administration of the drug.

Other treatments

Cardiovascular

Fluids

Crystalloid infusions [e.g. 0.9% saline or compound sodium lactate or Plasma-Lyte® (Baxter Healthcare Ltd, Northampton, UK)] were used for intravenous fluid resuscitation. Starch-containing colloid solutions were not used in view of evidence that they may be associated with adverse outcomes and increased rates of acute kidney injury.^{43–45} Gelatin-based solutions and human albumin solutions were allowed as alternative resuscitation fluids.

Fluid resuscitation was given based on repeated assessment of volume status, as detailed above.

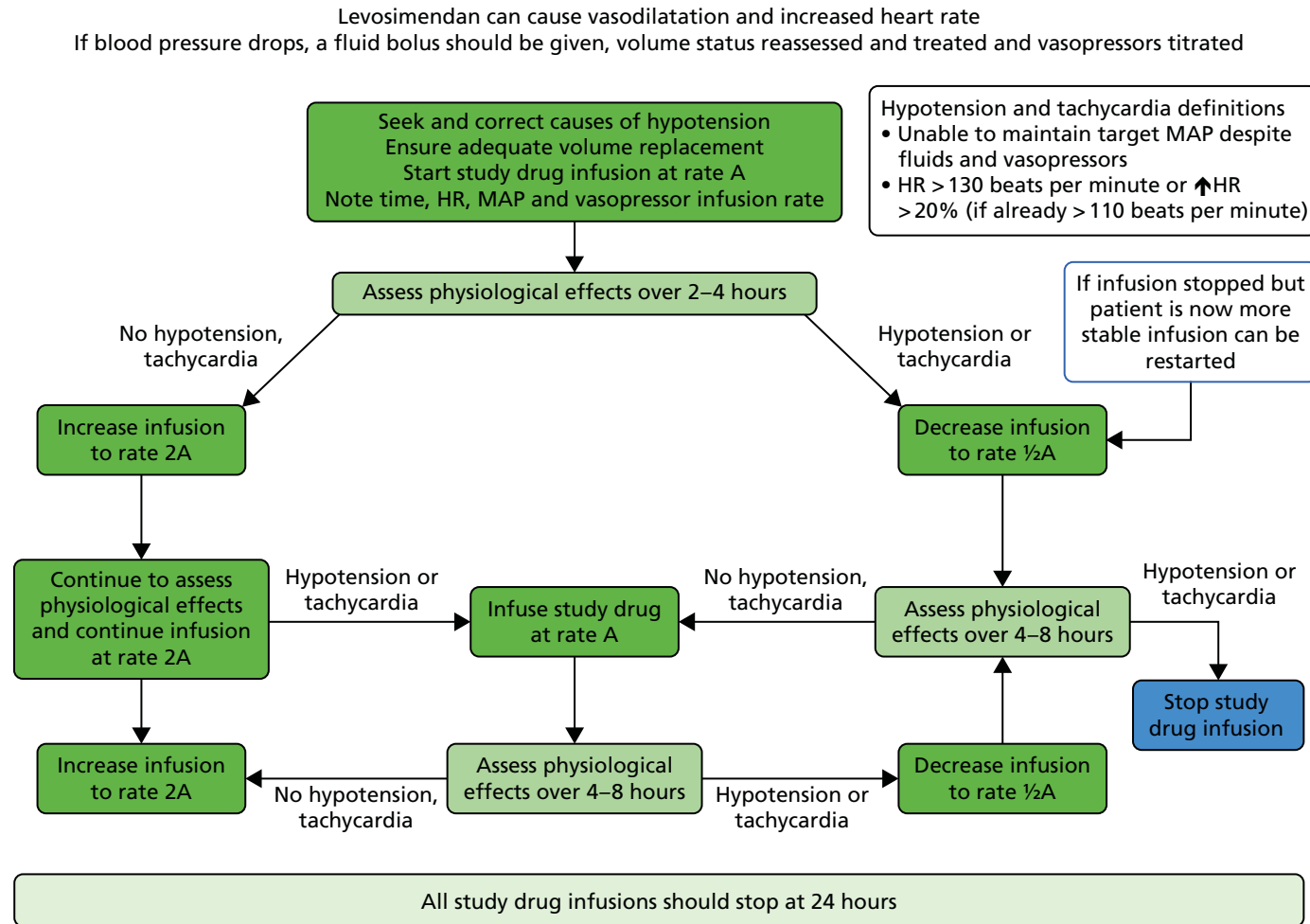


FIGURE 1 LePARDS trial drug infusion protocol. HR, heart rate.

Vasoactive drugs: vasopressors

Noradrenaline was the initial vasopressor of choice. After fluid resuscitation it was advised that noradrenaline should be titrated to maintain a target MAP of 65–70 mmHg. In individual patients a higher MAP target could be chosen, for instance if a patient was known to be hypertensive; similarly, in a normotensive patient a lower MAP target could be chosen. However, it was emphasised to investigators that the lowest dose of vasopressor to maintain an acceptable MAP to allow tissue perfusion should always be used.

Vasopressin, or any of its analogues, could also be used as an alternative vasopressor or in addition to noradrenaline.

Vasoactive drugs: inotropes

Additional inotropic agents could be used in either treatment group as clinically indicated (i.e. in the presence of low cardiac output after fluid resuscitation). Dobutamine was the inotropic agent of choice as per the Surviving Sepsis Campaign guidelines,⁴⁶ but other inotropes, including adrenaline or milrinone, were also allowed. Open-label levosimendan was not to be used in the LeoPARDS trial; however, after it was used at one site, the protocol was amended (v1.3) and it was clarified that open-label levosimendan was not to be used for any patients randomised into the LeoPARDS trial. There was a lack of evidence to recommend a set cardiac output/index target but, in general, an adequate cardiac output to ensure adequate oxygen delivery was to be maintained. It was also advised that a ScvO₂ of > 70% should be targeted in the early stages of septic shock management and that dobutamine (or any other inotropes) should be titrated down and patients weaned off once an adequate oxygen delivery was achieved.

Corticosteroids

It was advised that hydrocortisone should be used only for patients who were poorly responsive to vasopressors, that is, who were on high-dose vasopressors, as per the Surviving Sepsis Campaign guidelines.⁴⁶ Low doses were used (e.g. 200 mg/day of hydrocortisone in divided doses or as a continuous infusion) and doses were titrated down and patients weaned off once the shock resolved.

Ventilation

A lung-protective ventilation strategy was recommended, that is, 6–8 ml/kg of ideal body weight tidal volume, limiting plateau pressure to ≤ 30 cmH₂O, accepting permissive hypercapnia and ensuring adequate levels of positive end-expiratory pressure (PEEP) to prevent extensive lung collapse at the end of expiration.

High-frequency ventilation, neuromuscular blockade, inhaled nitric oxide, prone positioning and extracorporeal membrane oxygenation were all permitted as needed to manage severe hypoxaemia.

Renal support

Continuous venovenous haemo(dia)filtration was the renal replacement therapy (RRT) of choice and was used to treat the recognised complications of renal failure, that is, fluid overload, hyperkalaemia, symptomatic uraemia, drug accumulation and severe acid–base disturbance. High-volume haemofiltration for the management of sepsis (i.e. RRT not to treat kidney failure) was not advised.

Other management

All other general ICU management was based on the latest guidance from the Surviving Sepsis Campaign⁴⁶ and UK national critical care guidelines [e.g. the ventilator (<http://webarchive.nationalarchives.gov.uk/20120118171705/http://hcai.dh.gov.uk/files/2011/03/2011-03-14-HII-Ventilator-Associated-Pneumonia-FINAL.pdf>) and central line care bundles (<http://webarchive.nationalarchives.gov.uk/20120118171551/http://hcai.dh.gov.uk/files/2011/03/2011-03-14-HII-Central-Venous-Catheter-Care-Bundle-FINAL.pdf>)].

Inevitably, there were minor differences in management between different centres but using the Surviving Sepsis Campaign guidelines⁴⁶ ensured external generalisability of the results. Stratification of randomisation by treating centre also ensured balance of small ICU differences between treatment groups.

Dose modifications for toxicity

The study drug infusion protocol was followed to manage the expected pharmacodynamic effects of levosimendan, in particular vasodilatation and tachycardia.

Follow-up

Participants were followed up daily while on the ICU. Routinely collected clinical data (cardiovascular, respiratory and renal physiological variables as well as haematological, biochemical and microbiological blood test results) were recorded on a daily basis during this time.

Patients were also followed up to ascertain survival status at 28 days post recruitment, at hospital discharge and at 3 and 6 months post recruitment using local hospital clinical records or through the patients' general practitioners (GPs).

Laboratory evaluations

For blood and urine sampling, 25 ml of blood [\approx 12 ml in ethylenediaminetetra-acetic acid (EDTA) tubes for plasma, \approx 10 ml in plain tubes for serum, \approx 2.5 ml in PAXgene tubes for ribonucleic acid) and 10 ml of urine were collected on the day of inclusion (day 1), after 24 hours (day 2) and on days 4 and 6 while still on the ICU. Additional samples for drug-level assays were collected in select patients. Samples were separated locally, frozen according to standardised operating procedures and then sent to the co-ordinating centre in batches for storage and analysis.

End-point management

Outcomes

This section describes the primary and secondary outcomes; further detail of the statistical analysis can be found *Statistics and data analysis*.

Primary outcome

This trial was designed to fully explore the efficacy and mechanism of action of levosimendan. We therefore examined multiple organ failure, as measured by the Sequential Organ Failure Assessment (SOFA) score, as the primary end point. The SOFA score is the sum of scores relating to six organs. It comprises respiration, coagulation, liver, cardiovascular, renal and central nervous system components, each of which can have values from 0 (normal) to 4 (most abnormal), based on the most extreme values recorded each day.² In line with Table S3 in Myburgh *et al.*,⁴⁵ vasopressin treatment (any dose) was assigned a value of 3 for the cardiovascular component. We excluded the central nervous system from the daily calculation after randomisation as sedative drugs prevent an accurate daily assessment in this situation, and it has often been excluded in other septic shock clinical trials. Therefore, the SOFA score ranged from 0 to 20 on each day. *Table S1* (see *Report Supplementary Material 1*) shows the criteria for assigning values. For cardiovascular and renal scores, which have more than one contributing measure, the worst score was used. For the renal component, if urine output was not recorded on a particular day the SOFA component score was not considered missing and was based on the creatinine score only. For data recorded more than once a day on the case report form (MAP and drug dose for the cardiovascular component for the first 4 days), the worst value (i.e. the one leading to the highest SOFA score) was used for each day.

We calculated the SOFA score component attributable to the respiration, coagulation, liver, cardiovascular and renal components for each patient for each day that they were in the ICU, using measurements recorded in the daily data (see *Report Supplementary Material 1*). We calculated an overall patient SOFA

score for each day by adding the five components together. The mean SOFA score during the ICU stay was then calculated by adding the overall patient SOFA scores for all ICU days (up to a maximum of 28 days) and dividing by the number of days. The mean SOFA score in the ICU has been shown to be closely correlated with mortality and its predictive value was similar regardless of the length of stay.³ This helped solve the 'truncated by death' issue as all patients contributed scores while alive in the ICU.

Secondary outcomes

The SOFA score is a composite of several different organ failures and there may be differential effects of levosimendan in different organ systems. Therefore, to gain further insight into the mode of action of levosimendan, we also measured organ-specific outcomes.

Cardiovascular

In all patients we compared oxygen delivery between treatment groups using ScvO₂. This was measured and recorded at baseline and 6 and 12 hours and then 12-hourly in all patients with a jugular or subclavian central line for up to 96 hours and then daily to day 5 if the central line remained in situ.

In prespecified ICUs we aimed to measure cardiac output using calibrated devices [e.g. PiCCO (Maquet, Rastalt, Germany), LiDCOplus (LiDCO, London, UK), oesophageal Doppler or pulmonary artery catheter] in all patients included in the study. Cardiac output data were measured and recorded at baseline and 6 and 12 hours and then 12-hourly for up to 96 hours (as long as the device was clinically required).

Renal

In view of the importance of acute kidney injury as an independent determinant of outcome in septic shock and the previous data demonstrating a beneficial effect of levosimendan on kidney function in sepsis we also compared rates of renal failure using the Acute Kidney Injury Network (AKIN) definitions.⁴⁷ Each patient was categorised on a daily basis into the increasing stages of renal failure as shown in *Table S2* (see *Report Supplementary Material 1*), with stage 3 defining acute renal failure. The overall score is the worst of the scores from each component, with higher scores indicating poorer renal function.

Abdominal

As poor mesenteric perfusion and bowel ischaemia are believed to be major contributors to the pathogenesis of multiorgan failure in septic shock we analysed bilirubin levels (used to calculate the hepatic SOFA score) over time.

Respiratory

Recent evidence has suggested that levosimendan may also enhance calcium sensitivity in diaphragm muscle and thus improve diaphragm contractility.⁴⁸ In patients who required intubation and mechanical ventilation, time to successful liberation from mechanical ventilation was defined as:

- extubated with face mask, nasal cannulae or room air or
- T-piece/heat and moisture exchange (HME) filter breathing or
- tracheostomy mask breathing or
- continuous positive airway pressure (CPAP) breathing ≤ 5 cmH₂O without any pressure support or mandatory ventilation for 48 hours continuously.

We analysed the respiratory end points in three ways:

1. ventilator-free days, defined as:

- 0, for patients who died before extubation
- days to death minus ventilation days, for patients who died after extubation and before 28 days
- 28 minus the number of ventilation days, for patients who survived to 28 days

2. time to extubation, defined as the time to first successful extubation, with deaths treated as still being on the ventilator beyond the end of follow-up (28 days)
3. arterial partial pressure of oxygen (PaO_2)/fraction of inspired oxygen (FiO_2) ratio over time, using data collected at baseline and each day the patient remained ventilated in the ICU (up to 28 days).

Major acute kidney event

A major acute kidney event (MAKE) at a certain time point, for example day 28 or day 90, is a recently suggested alternative renal failure outcome.⁴⁹ We applied the version based on 28 days, in line with the daily data collection and other end points.

In the LeoPARDS trial, a patient was defined as having MAKE28 (MAKE 28 days after randomisation) if they experienced any of the following in the 28 days following randomisation:

- death
- need for RRT, excluding those who required RRT before randomisation
- sustained loss of kidney function, defined as acute kidney injury (AKI) stage 2 or 3 at day 28 or on ICU discharge if discharged before day 28.

Other secondary clinical outcomes

Other secondary clinical outcomes were:

- 28-day, ICU and hospital discharge, and 3 and 6-month survival
- ICU and hospital length of stay, and ICU-free days, defined as the number of days during the 28 days after randomisation that a patient was alive and not in the ICU
- duration of RRT, defined as the first day of RRT in the ICU to the last day of RRT, including any RRT received post discharge (all days in between were counted, whether or not the patient received RRT)
- days free from catecholamine therapy, defined as the number of days during the 28 days after randomisation that a patient was alive and did not receive any catecholamine therapy (dobutamine, adrenaline and noradrenaline)
- organ support data.

Secondary mechanistic outcomes

Serial blood and urine samples were collected from patients, as detailed earlier. A number of assays were considered for analysis including markers of acute kidney injury, myocardial dysfunction, inflammation and intestinal perfusion. Based on the clinical results the final biomarkers assayed included:

- Markers of myocardial dysfunction:
 - troponin, a widely used marker of myocardial injury and an early prognosticator of poor outcome in sepsis
 - serum brain natriuretic peptide [and N-terminal prohormone of brain natriuretic peptide (NT-proBNP)], demonstrated to be a reliable biomarker of ventricular dysfunction in septic patients and also a prognostic marker for a poor outcome.^{50,51}
- Biomarkers of systemic inflammation. The original plans had been to use a multiplex inflammatory biomarker bead assay. However, because of discontinuation of one such assay by the manufacturer, no reliable similar alternatives and issues with different dilutions required for different analytes, we selected five key biomarkers to study. These were:
 1. the pro-inflammatory cytokine interleukin (IL)-6
 2. the chemokines IL-8 and monocyte chemoattractant protein 1 [also known as chemokine ligand 2 (CCL2)] (levosimendan has been shown to reduce levels in sepsis)⁵²
 3. the anti-inflammatory cytokine IL-10

- soluble tumour necrosis factor receptor 1 (sTNFR1), an important soluble receptor that is one of the biomarkers used to characterise a hyperinflammatory phenotype in critical care, which is associated with higher mortality and potentially improved response to anti-inflammatory treatment.⁵³

In addition, samples were stored for subsequent analysis (e.g. genetics/proteomics/metabonomics) in future separately funded projects.

Plasma sample assay methodology

For troponin I, plasma samples were analysed in the clinical laboratories at Imperial College Healthcare NHS Trust. The high sensitive chemiluminescent microparticle immunoassay, Abbott Architect (Abbott Diagnostics, UK), was used. Samples were processed according to normal laboratory procedures.

N-terminal prohormone of brain natriuretic peptide

Plasma samples were quantified using a sandwich enzyme-linked immunosorbent assay (ELISA) kit (Abcam, Cambridge, UK). Samples, on their third freeze–thaw cycle (after measurement of biomarkers; see *Inflammatory biomarkers*), were run at a 1 : 4 dilution and, when required, were rerun at a 1 : 8 dilution.

Inflammatory biomarkers

Interleukin 6, IL-8, IL-10 and CCL2 were quantified using the ELLA multiplex assay (ProteinSimple™, San Jose, CA, USA). Samples were thawed at room temperature on the first freeze–thaw cycle and were then diluted by 1 : 3 to 1 : 8 in assay buffer and run as per the manufacturer's instructions. Positive controls of recombinant IL-6, IL-8, IL-10 and CCL2 standards were run alongside to ensure reproducibility. sTNFR1 was assessed using the ELLA Simple Plex assay. Samples were thawed at room temperature on the second freeze–thaw cycle, a minimum of 24 hours after the first cycle. Samples were then diluted 1 : 10 to 1 : 13 and run as specified by the manufacturer's instructions; positive controls were run alongside.

Pharmacokinetic/pharmacodynamic study

The first 80 patients enrolled in the study (with a full sample set collected) had an additional 3 ml of blood collected while on the ICU on days 2, 4, 6, 8, 10, 13 and 16, for assays of levosimendan and its active metabolites OR-1896 and OR-1855. The assays used liquid chromatography tandem mass spectrometry on a Thermo Finnigan TSQ Quantum™ Ultra device (Thermo Fisher Scientific, Waltham, MA, USA) and were carried out at LGC Group (Fordham, Cambridge, UK); this analysis was paid for by Orion Corporation. A non-compartmental analysis of pooled data was undertaken. The population area under the concentration–time curve (AUC) and the maximum concentration (C_{max}) were estimated. The AUC was estimated using the trapezoid rule and the C_{max} was estimated from visual inspection. We then compared the AUC and the C_{max} between patients with and without acute renal failure, acute liver failure and requirement for RRT and also with previous pharmacokinetic data from other studies. Analysis was performed using GraphPad Prism version 7.00 (GraphPad Software Inc., San Diego, CA, USA).

Data collection

Electronic case record form

Data management was carried out using the InForm ITM (Integrated Trial Management) system, a web-based data entry system that builds an Oracle database for each individual clinical trial. Trial data were captured on a bespoke web-based electronic case record form (eCRF) with built-in validation rules to identify data entry errors in real time and a full audit trail of data entry and changes. All those entering data were trained prior to start-up and given personal login details, with access to forms restricted according to site and role. The eCRF was designed in accordance with the requirements of the trial protocol, and access to the eCRF was password protected and included controlled level of access. A full list of the data collected on each form is provided in *Appendix 1*.

Pharmacovigilance definitions and procedures

Definitions

An adverse event (AE) was defined as any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of an IMP, whether or not considered related to the IMP.

An adverse reaction (AR) was defined as an untoward and unintended response to an IMP related to any dose administered. All AEs judged by either the reporting investigator or the sponsor as having a reasonable causal relationship to a medicinal product qualified as adverse reactions. The expression 'reasonable causal relationship' means to convey in general that there is evidence or an argument to suggest a causal relationship.

An unexpected AR was defined as an AR, the nature or severity of which is not consistent with the applicable product information (e.g. investigator's brochure for an unapproved investigational product or SmPC for an authorised product). When the outcome of an AR is not consistent with the applicable product information this AR should be considered as unexpected. Side effects documented in the SmPC that occur in a more severe form than anticipated are also considered to be unexpected.

A serious adverse event (SAE) or serious AR was defined as any untoward medical occurrence or effect that at any dose:

- results in death
- is life-threatening – refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe
- results in hospitalisation or prolongation of an existing inpatient hospitalisation
- results in persistent or significant disability or incapacity
- is a congenital anomaly or birth defect.

Medical judgement was exercised in deciding whether or not an AE/AR was serious in other situations. Important AEs/ARs that were not immediately life-threatening or that did not result in death or hospitalisation but may have jeopardised the subject or have required intervention to prevent one of the other outcomes listed in the definition above were also considered serious.

A suspected unexpected serious adverse reaction (SUSAR) was defined as any suspected AR related to an IMP that was both unexpected and serious.

Causality

The assignment of the causality was made by the investigator responsible for the care of a participant using the definitions in *Table 2*.

If there was any doubt about the causality, the local investigator informed the study co-ordination centre, which notified the chief investigator. In the case of discrepant views on causality between the investigator and others, all parties discussed the case, but the final decision was made by the local investigator.

Reporting procedures

Depending on the nature of the event the subsequent reporting procedures were followed. Any questions concerning AE reporting were directed to the study co-ordination centre in the first instance.

TABLE 2 Table of causality

Relationship	Description
Unrelated	There is no evidence of any causal relationship
Unlikely	There is little evidence to suggest that there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the participant's clinical condition, other concomitant treatment)
Possible	There is some evidence to suggest a causal relationship (e.g. because the event occurred within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the participant's clinical condition, other concomitant treatments)
Probable	There is evidence to suggest a causal relationship and the influence of other factors is unlikely
Definitely	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out
Not assessable	There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship

Non-serious adverse reactions/adverse events

All such toxicities, whether expected or not, were required to be recorded on the AE section of the eCRF form within 1 month of the form being due.

Serious adverse reactions/adverse events

Fatal or life-threatening SAEs were required to be reported on the day that the local site became aware of an event. The SAE form asked for the nature of the event, date of onset, severity, corrective therapies given, outcome and causality (i.e. unrelated, unlikely, possible, probably, definitely). The responsible investigator assigned the causality of the event. Additional information was sent to the study co-ordination centre within 5 days of notification if the reaction had not resolved at the time of reporting.

A SAE form was completed and entered into the eCRF, which automatically sent e-mail alerts to the chief investigator, the trial manager and the sponsor. However, relapse, organ failure and death related to sepsis (see following definitions), and hospitalisations for elective treatment of a pre-existing condition, did not need to be reported as a SAE.

Clinical outcomes

Clinical outcomes from sepsis were exempt from AE reporting, unless the investigator deemed an event to be related to the administration of the study drug. The following events were considered clinical outcomes:

- death related to sepsis
- cardiovascular failure, including the need for vasopressors/inotropes
- respiratory failure, including mechanical ventilation and acute lung injury
- hepatic failure
- renal failure, including the need for RRT
- haematological/coagulation failure, including thrombocytopenia.

Clinical details about these clinical outcomes were routinely collected on the case record form.

In relation to the study drug in this trial the following specific SAEs were recorded on the eCRF:

- myocardial infarction/acute coronary syndrome
- life-threatening arrhythmia (e.g. ventricular fibrillation, ventricular tachycardia or atrial fibrillation that leads to hypotension).

Suspected unexpected serious adverse reactions

In the case of SUSARs, the staff at sites were requested to complete the SAE eCRF (within 24 hours). The study co-ordination centre would then be notified by e-mail and would contact the local site for further information.

The study co-ordination centre would then notify the Medicines and Healthcare products Regulatory Agency (MHRA), the Research Ethics Committee (REC) and the sponsor of all SUSARs occurring during the study according to the following timelines: fatal and life-threatening – within 7 days of notification; non-life threatening – within 15 days. All investigators would have been informed of all SUSARs occurring throughout the study.

Local investigators were to report any SUSARs and/or SAEs as required by the protocol and study-specific Standard Operating Procedures (SOPs). There were no SUSARs in the LeoPARDS trial.

Annual safety reports

Annual safety reports were provided to the REC and MHRA, in accordance with clinical trial regulations, on the anniversary of the clinical trial authorisation each year. A total of three annual safety reports were submitted over the course of the trial.

Emergency identification of study medication/unblinding

Prior to any unblinding the site must have made a concerted effort to contact the chief investigator, his deputy or the trial manager.

Sealed envelopes containing the login and password for unblinding from the InForm ITM system were provided to participating ICUs. Sealed envelopes would not be opened under normal circumstances and the integrity of the envelopes was checked at every monitoring visit by the trial monitor.

To unblind a patient, the treating physician/principal investigator (PI) would contact the chief investigator or trial manager to discuss the need for, and obtain approval for, unblinding. If the need for unblinding was agreed, the treating physician/PI would open the sealed envelope. The enclosed login and password would then be used to unblind the patient using the InForm database. A File Note would then be completed detailing the circumstances of the unblinding. After the sealed envelope containing the unblinding instructions had been opened to unblind a patient, a new sealed envelope would be provided to the site by the trial manager/monitor. In the event that the InForm database was inaccessible, once the site and the study co-ordinating centre had agreed that unblinding was the best course of action, the Charing Cross Hospital on-call pharmacist could be contacted. The on-call pharmacist had access to the main trial unblinding list and would be able to expose the unblinded information.

One patient was unblinded in the LeoPARDS trial, after completion of the recruitment phase of the trial but before the statistical analysis of the data took place and just prior to database lock. In this case, the patient had been randomised into the trial, recovered from their episode of septic shock, left the ICU and later that year passed away in hospital. The patient's family had raised a complaint against the hospital trust and the care and subsequent death of the patient were being reviewed. The independent medical team had requested to know whether the patient had received the active drug or the placebo.

Data Monitoring and Ethics Committee

An independent Data Monitoring and Ethics Committee (DMEC) was set up to monitor progress, patient safety and any ethical issues involved in this trial. The DMEC reviewed trial progress, recruitment rates, event rates and safety data. A separate charter was drawn up defining its exact remit and criteria for reporting to the Trial Steering Committee (TSC). There were 6-monthly meetings of the independent DMEC.

Early discontinuation

Withdrawal criteria

Patients were free to withdraw at any stage of the study.

If a patient wished to withdraw from the study during the treatment period, the treating physician would no longer follow the trial protocol and the study drug would have been stopped. The patient's data may or may not have been included in the final analysis, according to the patient's wishes.

If a patient wished to withdraw from the study after the treatment period, no further data would be collected. The patient's data may or may not have been included in the final analysis, according to the patient's wishes.

Loss to follow-up

Patients were followed up post hospital discharge using hospital data provided by each research team and so loss to follow-up was low. If patients could not be traced using this system they were contacted through their GP.

Statistics and data analysis

All patients who were randomised were included in the analysis where possible, unless consent to use the data was withdrawn. For all outcomes, the primary analysis was carried out on an intention-to-treat (ITT) basis. The analysis of the primary outcome and safety data was repeated on an as-treated basis, excluding patients who did not receive any study drug. For all statistical modelling, the validity of the underlying assumptions was checked and any resulting alterations to the model specifications were documented. A full statistical analysis plan (SAP) was developed by the trial investigators and trial statisticians and reviewed and agreed by the TSC (see www.journalslibrary.nihr.ac.uk/programmes/eme/111408).

Missing data

For all measures, the number of patients with missing data was described by drug allocation. We considered whether or not the level and type of missing data had the potential to affect the estimates of interest by introducing bias or reducing precision.

Daily data were recorded as clinically indicated for up to 28 days while patients were in the ICU. A decision not to take a measurement usually reflected the clinical judgement that there had been no change in that variable or the patient was getting better. In the pilot study for the Vasopressin vs Noradrenaline as Initial therapy in Septic Shock (VANISH) trial,⁵⁴ only a small percentage (4%) of SOFA scores had missing values, and a similar level of missingness was expected for the LeoPARDS trial. For the previous pilot study, missing data were imputed as the last recorded value [last observation carried forward (LOCF)] as it was reasonable to assume that the data were not collected because no change was expected by the clinician. Therefore, for longitudinal outcomes we took the following approach:

- When there were only 1 or 2 consecutive days of data missing, or when the missing data occurred at the end of follow-up, measurements were imputed using the LOCF.
- Where there were ≥ 3 days of data missing, the average value of the last available and next available observation was used as the imputed value.
- If the first day was missing data the value from day 2 was taken.
- If the values for days 1 and 2 were both missing the baseline value was taken.

If the level of missingness was substantially higher than anticipated or the assumptions governing the approach described earlier were thought to be unreasonable, we planned to conduct sensitivity analyses using Bayesian methods.⁵⁵ This was required for the total SOFA score and its components; methods are described further in *Primary outcome*.

Data management

In addition to the validation rules built into the InForm system (see *Data collection*) and trial monitoring activities (see *Trial management, Monitoring*), the trial statistician conducted checks on the data exported from the InForm system. This included checking for discrepancies across forms, inconsistent dates and times, duplicate entries, outliers and missing data. Data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study, including the follow-up period.

Baseline data and other longitudinal monitoring

Baseline characteristics are described by arm and overall, using the median and interquartile range (IQR) for continuous variables and the number and percentage in each group for categorical variables. The following clinical measures were monitored at varying intervals during the ICU stay:

- MAP
- central venous pressure
- heart rate
- ScvO₂
- cardiac index
- platelet count
- bilirubin
- lactate
- arterial oxygen saturation (SaO₂)
- haemoglobin
- total intravenous fluid administered
- fluid balance
- PaO₂/FiO₂ ratio
- creatinine
- total urine output.

These measures are described longitudinally by treatment group using box plots (see *Figures 3–14*). Note that day 1 is slightly different from the other days as it ran from the time of randomisation to when the next ICU chart was completed, so day 1 is almost always < 24 hours. Subsequent days are all 24-hour periods until the day of discharge. Cardiovascular measures are additionally presented by subgroup as follows. The cardiac index is presented for two groups according to baseline cardiac output (lowest tertile and middle and highest tertiles combined) and ScvO₂ is presented for three groups according to baseline ScvO₂ [low (< 70%), normal (70–85%) and high (> 85%)]. We planned to investigate treatment differences over time for cardiac output, ScvO₂, bilirubin and PaO₂/FiO₂ ratio (see *Primary outcome, Subgroup analysis and Secondary outcomes*).

To assess how well the study drug was tolerated, the number of subjects receiving the study drug at 6, 12 and 24 hours was tabulated by dose level and treatment group. The number of subjects receiving noradrenaline and dobutamine, and the median and IQR of the dose received, are described by treatment group.

Safety data

Adverse events were summarised by seriousness, relationship to study medication and treatment group. This information was shown as the number of AEs (subjects could contribute more than one AE) and the number of subjects (each subject is shown only once, using the AE with the most serious classification and the highest level of causality).

Using the descriptions recorded on the AE and SAE report forms, each SAE was assigned to one of the following categories:

- myocardial infarction/acute coronary syndrome
- life-threatening arrhythmia (e.g. ventricular fibrillation, ventricular tachycardia or atrial fibrillation that leads to hypotension)
- other.

Furthermore, SAEs were grouped and tabulated by treatment according to organ system:

- cardiovascular/circulatory
- digestive/gastrointestinal
- nervous
- respiratory
- urinary/excretory
- musculoskeletal
- skin/hair/nails
- other.

Adverse events by category and organ system were reported for each treatment group.

Primary outcome

The primary outcome was the mean SOFA score on the ICU up to 28 days after randomisation. The score was calculated as described in *End-point management, Primary outcome*.

Exploratory analysis

To explore the distribution of the primary outcome, box plots of the total SOFA score by day and treatment arm were produced, along with bar plots by component up to day 7.

The calculation of the mean SOFA score requires a total SOFA score for each day that the patient is in the ICU. To assess the level of missingness, we tabulated the percentage of subjects with at least one missing score during the ICU stay and the percentage of daily scores that were missing. This was carried out for the total SOFA score and for each component separately.

Main analysis of the primary outcome

The mean total SOFA score and its components are presented as means and standard deviations (SDs) and medians and IQRs by treatment group. The treatment difference was the unadjusted mean difference in the total mean SOFA score between the levosimendan arm and the placebo arm, with a 95% confidence interval (CI). Although randomisation was stratified by site, as there were a lot of sites, many of which recruited only a handful of patients, including site as a covariate did not appear to be analytically sensible. Instead, we allowed for any correlation of outcomes between patients at the same site by including a random intercept in the secondary analysis (see *Adjusted analysis*). Histograms and Q–Q plots were used to check the assumption that the distribution of the total SOFA score was approximately normal. As this was not the case, the CIs for the means in the primary analysis were calculated using bootstrapping, applying the percentile method with 100,000 samples. In addition, the difference in medians is also presented and for the total SOFA score the square root transform was applied, although lack of a back transform meant that this result was less clinically meaningful. To provide quality assurance, the main analysis of the primary outcome was repeated by another statistician at the ICTU using different software (Stata, version 13.1; StataCorp LLC, College Station, TX, USA), including calculation of the SOFA scores from the raw data.

Sensitivity analysis of the primary outcome

We conducted a series of sensitivity analyses, as detailed in the following sections.

Omitting the cardiovascular component

The main analysis was repeated but omitting the cardiovascular component from the SOFA score. The cardiovascular component of the SOFA score is based largely on the dose of catecholamine given to treat shock. Levosimendan has a similar mode of action (improving cardiovascular function) but is not included within the cardiovascular SOFA score. It was expected that the patients treated with levosimendan would need less catecholamine therapy. As the levosimendan therapy is not recorded within the SOFA score this might have resulted in a spurious lowering of the cardiovascular component of the SOFA score.

Adjusted analysis

Regression models were used to check whether or not the main analysis result was sensitive to adjustment for age and severity of illness (as indicated by the APACHE II score at baseline). Adjustment for age and APACHE II score is typically included in the analysis of data from critical care trials. A random intercept model was used to check whether or not the results were sensitive to clustering by ICU.

Bayesian analysis

The overall level of missingness across SOFA components was 6.2%, higher than the 4% expected. LOCF methods may underestimate uncertainty so, as specified in the SAP (see www.journalslibrary.nihr.ac.uk/programmes/eme/111408), to check the robustness of the primary result a sensitivity analysis was conducted, implementing Bayesian models using Markov chain Monte Carlo (MCMC) methods that differed from the main analysis in two ways. First, the imputed values varied with each MCMC iteration, reflecting the additional uncertainty of missing values. Second, the assumptions governing the imputed values differed. An autoregressive process resulting in imputed values similar to the adjacent values was used and assumptions reflecting the clinical expectation that missing values were more likely to be normal were incorporated. Similar to the main analysis, a bootstrap approach was used because of the non-normal distribution of the daily total SOFA scores, with a separate bootstrap sample taken at each MCMC iteration. Further details of the methods are provided in *Report Supplementary Material 1*.

Post hoc sensitivity analysis

Any differential effect of treatment on ICU discharge or death could affect the comparison of mean SOFA scores between groups. Two further imputation approaches were conducted to investigate this. First, the last recorded score was carried forward to all days after ICU discharge or death, up to day 28. Second, all days alive outside the ICU were assigned the minimum possible score of zero and all days dead were assigned the maximum score obtained by the patient in question. Note that these analyses were conducted post hoc in response to comments from reviewers at publication stage.

Subgroup analysis

The following a priori-defined subgroup analyses were carried out for the primary outcome:

- cardiac index – using the baseline cardiac index, we split patients into two groups: lowest tertile and middle and highest tertiles combined
- ScvO₂ – using baseline ScvO₂, we split patients into three groups: low (< 70%), normal (70–85%) and high (> 85%)
- lactate – using baseline lactate level, we split patients into two groups: normal (≤ 2 mmol/l) and raised (> 2 mmol/l)
- noradrenaline – using baseline noradrenaline level, we split patients into two groups: above and below the median.

The results are presented in the same way as for the main analysis of the primary outcome. In addition, a forest plot is used to display the results and, as a post hoc analysis, a permutation test was used to investigate subgroup heterogeneity.⁵⁶

Secondary outcomes

Cardiovascular

For both ScvO₂ and the cardiac index, we used hierarchical regression models to take into account the structure in the data, incorporating random effects to allow for repeated measurements from individuals. To allow the effect of treatment to vary over time, treatment, time and the interaction between treatment and time terms were included. Mathematical and implementation details are provided in the SAP (see www.journalslibrary.nihr.ac.uk/programmes/eme/111408).

For the cardiac index the difference between groups was summarised as the difference between the AUCs on the log scale. We also calculated the ratio comparing levosimendan and placebo on the original scale, at each time point and averaged over all time points using the geometric mean. In the SAP (see www.journalslibrary.nihr.ac.uk/programmes/eme/111408) we planned two analysis populations: first, patients in the subgroup of ICUs in which $\geq 75\%$ of the trial participants were monitored and, second, all patients, with the latter yielding a larger sample although possibly not representative of the whole population. As only two ICUs contributing 19 patients met the criterion for the first analysis, only the second analysis was carried out.

Renal

For the renal assessment, we chose to focus on renal failure on day 14. This choice was based on simulation with the aim of maximising power to detect a treatment difference (see *Power calculations*). Full details of the simulation study were included in the SAP (see www.journalslibrary.nihr.ac.uk/programmes/eme/111408). On day 14, each patient was categorised into increasing stages of renal failure according to the international AKIN definitions (0, 1, 2, 3) (see *Table S2, Report Supplementary Material 1*). If the intervention reduced death, excluding those who had died by day 14 might lead to the appearance of increased illness severity in the treatment group as more patients would have been alive but likely to have had a high AKIN score. Patients who died on or before day 14 were included and classified as stage 4, as death is a worse outcome than renal failure. Patients who were discharged alive from the ICU were assumed to have a score of 0 unless they received RRT post discharge.

This created five-group ordinal categorical data, that is, 4 is worse than 2 but not necessarily twice as bad. Rather than modelling the (log) odds of having the event, as for binary data, we modelled the (log) odds of being a category j or higher, with $j = 1, 2, 3$ or 4 for the renal failure data. The result of interest is the ratio of these odds in the levosimendan and placebo arms. We can assume that the OR is constant across values of j (a proportional odds model) or independent across j (a non-proportional odds model). Alternatively, we can constrain the ORs to be constant over some but not all values of j (a constrained proportional odds model). This approach was consistent with our expectation that levosimendan would have a greater impact on the transition between the different levels of renal injury/failure (scores 0–3) than between these stages and death (score 4). This assumption was explored using simulation as documented in the SAP (see www.journalslibrary.nihr.ac.uk/programmes/eme/111408) and was found to give the best fit using simulated data with parameters derived from published data and data from the three ICUs within the Imperial College Healthcare NHS Trust. Therefore, the ordinal categorical data for renal failure on day 14 were modelled using a constrained non-proportional odds model, such that the log ORs are assumed to be constant across all of the cumulative probabilities except the last, death. We additionally present results from the proportional and non-proportional models for comparison.

We also present the number and proportion of patients receiving any RRT and the median and IQR for the duration of RRT, separately for all patients and for those receiving some RRT.

Respiratory

Ventilator-free days

Because of the non-parametric distribution of ventilator-free days, we present the number and proportion of patients in each treatment group with no days free and with 28 days free, as well as the absolute

difference in proportions and 95% CI. As a summary of the whole population, we present the medians and IQRs, testing the difference between groups using a Mann–Whitney test and presenting the absolute difference in medians with the bootstrapped 95% CI.

Time to extubation

Time to extubation was illustrated using Kaplan–Meier plots and analysed using a Cox proportional hazards model. We present both an unadjusted model and a model adjusted for age, APACHE II score and ICU effects, similar to the regression analysis for the primary outcome.

Arterial partial pressure of oxygen/fraction of inspired oxygen ratio

An appropriate model for the PaO_2/FiO_2 ratio is a patient-specific random-effects model to allow for repeated measurements per patient. It is plausible that respiratory function is correlated with dropout as a result of death or discharge, potentially leading to biased estimates from the longitudinal model. Therefore, we chose to model the longitudinal respiratory data jointly with survival in order to explicitly acknowledge the underlying relationships and minimise the risk of bias. Following the proposal of Henderson *et al.*,⁵⁷ the patient-specific random effects are linked to the survival model using three parameters modelling the association between the survival time and (1) the intercept, (2) the slope and (3) the current subject-specific random effects. Based on data from the VACS (Vasopressin And Corticosteroids in Septic Shock)⁵⁸ and VANISH⁵⁴ trials, for the PaO_2/FiO_2 ratio we chose a hierarchical model with correlated intercepts and slopes, assuming a linear relationship with time, and for the survival data we chose a Weibull model. Full methods are provided in the SAP (see www.journalslibrary.nihr.ac.uk/programmes/eme/111408).

A number of amendments were made in order to achieve model convergence: PaO_2/FiO_2 ratio measurements, including at baseline, were mean-centred and standardised, the effect of the baseline measurement was included as a separate parameter, and the $N(0,100)$ prior was used for the parameters linking the longitudinal and survival models and for the intercept and treatment effect in the survival model. This prior has a narrower range than the $N(0,10000)$ prior proposed in the SAP (see www.journalslibrary.nihr.ac.uk/programmes/eme/111408), but it still supports a wide range of reasonable values.

We report:

- the probability that the PaO_2/FiO_2 ratio improves more quickly for patients receiving levosimendan than for patients receiving placebo during their stay in the ICU
- the mean change per day in PaO_2/FiO_2 ratio for patients receiving levosimendan
- the mean change per day in PaO_2/FiO_2 ratio for patients receiving placebo
- the difference in the mean change in PaO_2/FiO_2 ratio between patients receiving levosimendan and those receiving placebo over (1) 1 day, (2) 7 days (the length of time that the active metabolite of levosimendan is present in plasma) and (3) the mean length of stay (calculated across both arms).

A series of sensitivity analyses were carried out by repeating the above analysis as follows:

- restricting the PaO_2/FiO_2 ratio data to (1) the first 7 days, (2) the first 14 days and (3) the first 21 days
- incorporating age and APACHE II score at baseline into the measurement and survival models as explanatory variables
- adjusting for ICU effects by adding a third level to the measurement model (PaO_2/FiO_2 ratios modelled within patients within ICUs).

Liver

Analogous to the analysis of the PaO_2/FiO_2 ratio, we jointly modelled bilirubin level as a patient-specific random-effects model and death using a survival model. However, unlike the PaO_2/FiO_2 ratio, bilirubin has a markedly skewed distribution, so we used a logarithmic transform to better comply with the assumption of normal errors. Similar modifications were made to achieve convergence: the log baseline measurements were mean-centred and standardised, the effect of the baseline measurement was included as a separate

parameter and, in the three sensitivity analyses restricted to data from the first 7, 14 and 21 days, a $N(0,100)$ prior was used for the parameters linking the longitudinal and survival models.

Other secondary clinical outcomes

For the outcome of MAKE28, we present the number and proportion of patients in each arm experiencing the event. The treatment difference was described using the risk difference and 95% CI and using logistic regression adjusted for age, APACHE II score and ICU effects.

For survival outcomes we present Kaplan–Meier plots and describe the treatment difference using the hazard ratio from Cox regression models, both unadjusted and adjusted for age, APACHE II score and ICU effects. We also present the number and proportion of patients in each arm experiencing the event, together with the risk difference and 95% CI. This was repeated for the subgroups for the primary outcome (see *Primary outcomes, Subgroup analysis*) as a post hoc analysis.

For ICU-free days, we took a similar approach as for ventilator-free days, presenting the number and proportion of patients in each treatment group with no free days and the absolute difference in proportions with the 95% CI. As a summary of the whole population, we present the medians and IQRs, testing the difference between groups using a Mann–Whitney test and presenting the absolute difference in medians with the bootstrapped 95% CI. We analysed days free of catecholamine therapy in a similar manner but did not plan a formal hypothesis test.

The median and IQR for length of stay and organ support days are described by treatment arm and for the whole trial population. Length of stay was analysed separately for survivors and non-survivors. No formal statistical comparisons or tests were conducted.

Biomarker data

Seven biomarkers were analysed: one marker of myocardial dysfunction (NT-proBNP), one marker of myocardial injury (troponin I) and five markers of the systemic inflammatory and anti-inflammatory response (IL-6, IL-8, IL-10 and CCL2 and sTNFr1). Samples were taken at baseline (date of study entry) and on days 2, 4 and 6. The number of measurements at each time point was described and box plots produced over time.

The mean total SOFA score (both including and excluding the cardiovascular component), the mean SOFA component scores and 28-day mortality were analysed by subgroups of NT-proBNP and troponin values. The study population was split in two ways:

1. normal compared with high values [upper limit of normal values: 34 ng/l for troponin (Imperial College Healthcare NHS Trust) and 2000 pg/ml for NT-proBNP, according to NICE guidelines⁵⁹]
2. below and above the median value in the study population.

For all biomarkers, we used Bayesian hierarchical regression models to investigate changes in biomarker levels over time and whether or not trajectories differed for levosimendan and placebo patients. A random intercept term was used to allow for the correlation of multiple measures per patient, with a treatment × time interaction to model differing trajectories in the treatment groups, and adjustment was made for the baseline values of the biomarker. All biomarkers, including baseline values, were log transformed to better comply with the assumption of normal error terms. As this analysis was exploratory we present models both with and without the treatment × time interaction. Sensitivity analysis was performed adjusting for age and APACHE II score at baseline and allowing for clustering by ICU with a further level of random effects. Full details can be found in the SAP (see www.journalslibrary.nihr.ac.uk/programmes/eme/111408) for biomarker analysis.

To describe the effects of levosimendan we present:

- the estimated change in biomarker levels per day for levosimendan and placebo patients
- the probability of a faster reduction in biomarker levels in the levosimendan group than in the placebo group
- the estimated treatment difference on days 2, 4 and 6.

Power calculations

Our sample size was 500 patients. This would provide > 90% power to detect a 0.5-point difference in mean SOFA score assuming a SD of 1.5.³ In this previous validation study a 1-point rise in mean SOFA score was associated with a significant mortality increase (mean SOFA score 2.1–3.0 = 20% mortality rate, 3.1–4.0 = 36.1% mortality rate and 4.1–5.0 = 73.1% mortality rate; OR 3.06, 95% CI 2.36 to 3.97). We recruited an additional 3% (16 patients) to account for the potential loss to follow-up and withdrawal of consent, as seen in previous UK ICU trials.⁶⁰ Hence, 516 patients were recruited, with the goal of obtaining approximately 250 evaluable patients per treatment arm.

Renal outcome

Because of the non-parametric nature of the renal outcome data, calculation of the power for the renal outcome was undertaken using simulation with multistate modelling. The event rates and frequencies of moving between the states of no renal failure, developing renal failure, recovery and death were informed from mortality and organ failure data from the VASST (Vasopressin And Septic Shock Trial),^{61–63} the CORTICUS (Corticosteroid Therapy of Septic Shock) trial,⁶⁴ local data from the three adult ICUs within Imperial College Healthcare NHS Trust and the recently completed septic shock study by Gordon *et al.*⁵⁸

Patients were classified according to the three stages of renal dysfunction as per the AKIN definitions⁴⁷ and data were analysed as ordinal categorical data. Death, which is clearly the worst outcome, was taken into account by classifying it as stage 4, as detailed earlier. We compared the ordinal categorical data for death with acute renal failure at day 14. Based on a range of different transition probabilities we have 65–90% power to detect a 25–35% improvement with levosimendan (previous studies have demonstrated a 24–64% improvement in renal function).

Cardiovascular outcome

For oxygen delivery, 500 patients would provide > 95% power to detect a 5% difference in ScvO₂, assuming a SD of 15%.⁶³

Respiratory outcome

More than 90% of patients who have septic shock will require intubation and mechanical ventilation. Time to final successful liberation from mechanical ventilation was compared between the two treatment groups using survival analysis. A total of 450 patients (90% of 500) would provide 80% power to detect a hazard ratio of 1.4, based on the assumption that, overall, 63% of patients will be successfully liberated from mechanical ventilation by day 28.

The DMEC, including an independent statistician, was instructed within its charter to check these planning assumptions and undertake an interim sample size review.

Treatment

Investigational medicinal product details

Orion Corporation Orion Pharma supplied the study drugs for this trial (Table 3).

TABLE 3 Investigational medicinal product details

Drug name	Dosage	Description
Levosimendan	0.05–0.20 µg/kg/minute	Supplied as 8-ml vials with a nominal filling volume of 5 ml containing 12.5 mg of levosimendan and inactive ingredients (povidone, anhydrous citric acid and anhydrous ethanol)
Placebo	0.05–0.20 µg/kg/minute (equivalent)	Supplied as 8-ml vials with a nominal filling volume of 5 ml containing riboflavin, sodium phosphate, anhydrous ethanol and water

Labelling, storage and dispensing

Orion Corporation were responsible for assuring that the quality of all IMPs was adequate for the duration of the trial and in compliance with the Good Manufacturing Practice standards (www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_001205.jsp&mid=WC0b01ac0580027088).

Levosimendan and the matched placebo were imported from Finland. All study drugs were packaged, labelled to meet the MHRA requirements and distributed to sites by Victoria Pharmaceuticals. The study co-ordination centre kept accurate records of supply to trial centres and destruction of unused IMPs at the end of the trial.

It was each trial centre's responsibility to ensure that accurate records of IMPs dispensed and returned were maintained and reported to the study co-ordination centre. It was the PI's responsibility to ensure that accurate records of IMP prescriptions were maintained. The study co-ordination centre tracked supplies of IMPs via information from Victoria Pharmaceuticals and site IMP tracking documents. At the completion of the trial, the study co-ordination centre, via the trial monitor, ensured the destruction of all returned dispensed IMPs (after close-out and before archiving).

Accountability

Hospital pharmacies were responsible for recording study drugs dispensed to the ICU. Preparation of all drug infusions was recorded on the ICU drug accountability form and drug administration was recorded on each patient's prescription chart. The study pharmacies included a sheet on which the fate of all ampoules was recorded (infused, opened but not infused, unused). At the end of the study all remaining unused drugs were returned to the hospital pharmacies for recording and destruction.

Administrative matters

Source data

Source documents included original documents related to the trial, medical treatment and the history of the participants.

Electronic data capture

The principal means of data collection from participant visits was electronic data capture (EDC) via the internet. Data were entered into the EDC system by site personnel. All source data recorded on the eCRF were signed by the PI or his or her appropriate designee. All changes made following the electronic signing had an electronic audit trail with a signature and date. Specific instructions and further details were outlined in the CRF manual.

Trial management

The UK Clinical Research Collaboration (UKCRC)-registered ICTU was responsible for trial management, quality assurance, trial statistics and development and maintenance of the trial database. A dedicated trial manager and clinical trial monitors were appointed through the ICTU to oversee the day-to-day management and monitoring of the project from set-up to close.

Trial sponsor

The sponsor of the trial was Imperial College London. Imperial College London signed a clinical trial agreement with each of the participating centres prior to the start of recruitment at each centre.

Ethics considerations

The trial was conducted in accordance with principles of the Declaration of Helsinki⁶⁵ on research involving human subjects. The study protocol, patient information sheet and consent form were submitted to the REC prior to the start of the study and a favourable opinion was obtained on 26 April 2013.

Consent

Patients, personal legal representatives and professional legal representatives were given the patient information sheet and sufficient time to consider participation, and discussed the trial with the research staff prior to consent and enrolment. Full written informed consent was taken using the ethics-approved consent form.

Research governance

The trial was carried out in accordance with the NHS Research Governance Framework (www.hra.nhs.uk/planning-and-improving-research/policies-standards-legislation/uk-policy-framework-health-social-care-research/) and local NHS permission was granted by the research and development departments at each participating site prior to recruitment commencing.

Regulatory requirements

As a randomised trial of an IMP, the LeoPARDS trial was conducted in accordance with the European Clinical Trials Directive (https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-dir_2001_20/dir_2001_20_en.pdf) and the Medicines for Human Use (Clinical Trials) Regulations 2004 (www.legislation.gov.uk/ukxi/2004/1031/pdfs/ukxi_20041031_en.pdf), as well as the International Conference of Harmonisation (ICH) Good Clinical Practice (GCP) guidelines (www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6/E6_R1_Guideline.pdf). The trial received clinical trial authorisation from the MHRA on 28 August 2013 and was registered in the European Community with a EudraCT number of 2012-005159-18.

Trial registration

The trial was registered on the ClinicalTrials.gov clinical trial database with the following reference: ISRCTN12776039.

National Institute for Health Research Clinical Research Network portfolio

The LeoPARDS trial was adopted on the National Institute for Health Research (NIHR) Clinical Research Network (CRN) portfolio/Central Portfolio Management System with a UKCRN ID number of 15139. Accrual data were uploaded onto the NIHR CRN database on a monthly basis.

Summary of protocol amendments

A summary of the amendments made to the trial protocol following approval of the first version of the document by the REC and MHRA is provided in *Table 4*.

Trial committees

Trial Management Group

The Trial Management Group (TMG) was set up by the chief investigator and was established to discuss recruitment and other practical aspects of the trial. The TMG met 22 times over the course of the trial, on 5 October 2012, 12 November 2012, 6 December 2012, 14 January 2013, 11 February 2013, 11 March 2013, 8 April 2013, 13 May 2013, 10 June 2013, 12 August 2013, 9 September 2013, 14 October 2013, 11 November 2013, 9 December 2013, 19 May 2014, 28 July 2014, 13 October 2014, 5 December 2014, 11 March 2015, 22 June 2015, 13 October 2015 and 19 May 2016.

TABLE 4 Summary of protocol amendments

Protocol version	Amendment	Description	Location
1.1	Substantial amendment 08	<p>Clarification of exclusion criteria: previous:</p> <p><i>Severe hepatic impairment (Child–Pugh class C)</i></p> <p>New:</p> <p><i>Severe chronic hepatic impairment (Child–Pugh class C)</i></p> <p>Clarification of follow-up: previous:</p> <p><i>Patients will also be followed up to ascertain survival status at 28 days post recruitment, at hospital discharge, and at 3 and 6 months post recruitment using The Health and Social Care Information Centre [NHS Digital]</i></p> <p>New:</p> <p><i>Patients will also be followed up to ascertain survival status at 28 days post recruitment, at hospital discharge, and at 3 and 6 months post recruitment using The Health and Social Care Information Centre [NHS Digital] or via their GP</i></p> <p>Improve consistency in secondary outcomes, cardiovascular: previous:</p> <p><i>This should be measured and recorded at baseline, 6 and 12 hours and then 12 hourly in all patients with a jugular or subclavian central line for up to 96 hours and then daily to day 7 if the central line remains in situ</i></p> <p>New:</p> <p><i>This should be measured and recorded at baseline, 6 and 12 hours and then 12 hourly in all patients with a jugular or subclavian central line for up to 96 hours and then daily up to and including day 4 if the central line remains in situ</i></p> <p>Typo, clarification in appendix infusion guideline: previous – diagram did not display possibility of restarting drug; new – diagram now displays possibility of restarting the study drug if the patient has stabilised</p> <p>Clarification: 14 appendix 2 – echocardiographic substudy: new – 14 appendix – echocardiographic substudy</p>	<p>p. 12, 5.1.3</p> <p>p. 17, 5.2.5</p> <p>p. 17, 5.3.2</p> <p>p. 33, 5.2.5 13</p> <p>pp. 35 and 36</p>
1.2	Minor amendment 03	Restart of pharmacokinetic substudy to collect 80 full sample sets	p. 18, 5.3.2
1.3	Minor amendment 04	Clarification that open-label levosimendan is not permitted	p. 16, 5.2.4
1.4	Minor clarification to the protocol	Additional wording to clarify the collection of follow-up data from LeoPARDS trial patients	p. 17; p. 33, 13; p. 23, 7.3

The TMG membership is listed below:

- Professor Anthony Gordon
- Professor Danny McAuley
- Professor Mervyn Singer
- Professor Deborah Ashby
- Professor Gavin Perkins
- Dr Rob Orme
- Dr Shalini Santhakumaran
- Dr Alexina Mason
- Dr Farah Al-Beidh

- Dr Jonas Lexow
- Mrs Mary Cross
- Mrs Janis Best-Lane
- Miss Ashley Campbell.

Trial Steering Committee

A TSC was established to oversee the conduct of the study. The TSC met five times over the course of the trial, on 11 October 2013, 3 June 2014, 29 January 2015, 9 October 2015 and 15 June 2016. Copies of the minutes from each meeting were sent to the funder, the Efficacy and Mechanism Evaluation (EME) programme of the NIHR. The TSC approved the trial protocol prior to the start of the study and received regular recruitment reports throughout the duration of the trial.

The TSC membership is listed below:

- independent members:
 - Dr Simon Baudouin – chairperson
 - Dr Andrew Rhodes – independent member
 - Dr Phil Hopkins – independent member
 - Mrs Catherine White, ICU Steps – patient representative
 - Mr Simon Denegri – independent member
- non-independent members:
 - Professor Anthony Gordon – chief investigator
 - Mrs Mary Cross – operations manager, ICTU
 - Dr Rob Orme – member
 - Professor Deborah Ashby – co-investigator and statistician
 - Dr Alexina Mason – trial statistician
 - Ms Nabila Youssouf – trial sponsor representative.

Data Monitoring and Ethics Committee

An independent DMEC was established to review SAE reports and any ongoing safety issues. The DMEC meetings took place on 26 November 2013, 28 April 2014, 9 December 2014, 26 March 2015, 22 September 2015 and 15 June 2016.

The first DMEC meeting to agree the charter outlining operational details and responsibilities took place early in the trial, on 26 November 2013. The DMEC provided feedback reports for each meeting to the chairperson of the TSC and these were reviewed at subsequent TSC meetings as applicable.

The DMEC membership is listed below:

- independent members:
 - Professor Julian Bion – independent member chairperson
 - Dr Duncan Wyncoll – independent member
 - Dr Graeme MacLennan – independent statistician
- non-independent attendees (in attendance for open sessions only):
 - Professor Anthony Gordon – chief investigator
 - Mrs Mary Cross – operations manager, ICTU
 - Professor Deborah Ashby – co-investigator and statistician
 - Dr Alexina Mason – senior trial statistician (in attendance for closed sessions)
 - Dr Shalini Santhakumaran – trial statistician (in attendance for closed sessions).

Monitoring

A monitoring plan was devised based on risk analysis and was described in detail in the monitoring manual by the trial manager. Trial monitors visited all sites and facilities where the trial took place to ensure compliance with the protocol, GCP and local regulatory compliance, and patient safety and accurate data collection and reporting. Monitoring visits were dependent on the rate of recruitment and number of participants recruited per site. Regular communication with sites by telephone, mail and e-mail was undertaken. Training sessions were organised for the investigators and all site staff at the beginning of the trial and then as appropriate. Initiation visits were completed at all trial centres prior to the recruitment of participants, and consisted of a review of protocol and trial documents, training with respect to trial procedures (informed consent, SAE reporting, inclusion and exclusion criteria), and a review of the recruitment strategy, site facilities and equipment, and essential document receipt, collection and filing, and archiving and inspection. Copies of the trial-specific procedure manuals and related documents were given to the investigators and research team. The approved version of the protocol was followed at all times and any significant protocol deviations were documented on a protocol violation form and submitted to the study co-ordination centre as soon as possible. The investigators allowed the monitors to:

- inspect the site, facilities, IMP management and materials used for the trial
- meet all members of the team involved in the trial and ensure that all staff working on the trial were experienced, appropriately trained and had access to review all of the documents relevant to the trial
- have access to the eCRFs and source data
- discuss trial progress and any issues with the investigator and site staff on a regular basis.

The monitor ensured that:

- all participant records were inspected for confirmation of existence, eligibility and informed consent
- there was adherence to the protocol, including consistency with inclusion/exclusion criteria
- there was GCP and regulatory compliance
- trial documentation was complete and up-to-date (e.g. correct versions of documents being used, source data captured) and relevant documents were collected for the Trial Master File (TMF)
- the eCRFs had been completed correctly and accurately and all entries corresponded to data captured in source documents
- the IMP accountability records were in order (receipt, dispensing and destruction), storage was under appropriate conditions and secure expiry dates were being checked and adhered to, and dispensing was carried out according to the protocol and trial procedures.

All information dealt with during such visits was treated as strictly confidential. At the end of the trial, close-out visits were performed by the monitors after the final participant visit had been completed and prior to database lock. During this visit the monitors verified that all trial close-out activities had been completed: all queries had been resolved, missing data had been completed, monitoring had been completed, archiving arrangements were in place, IMP accountability was complete and all used and unused IMPs had been destroyed, the Investigator Site File (ISF) was completed and TMF documents had been collected and the end-of-trial notification had been completed.

Patient and public involvement

Two patient and public involvement representatives sat on the TSC and provided input from a patient perspective at trial meetings. Both representatives reviewed and provided feedback on all of the project documents prior to the ethics and regulatory submissions, and their comments were incorporated.

Quality control and quality assurance

Quality control was performed according to ICTU internal procedures. The study was audited by a quality assurance representative of the sponsor. All necessary data and documents were made available for inspection.

Audits and inspections

The study may be subject to inspection and audit by Imperial College London under its remit as sponsor, the study co-ordination centre and other regulatory bodies to ensure adherence to GCP.

Chapter 4 Results

Screening and participant flow

In total, 2382 patients were screened at the participating hospitals/centres over the duration of the trial. Of the 824 patients meeting the eligibility criteria, 308 declined to participate and 516 were randomised to the trial. The flow of patients is summarised in *Figure 2*, including the numbers of patients screened, randomised and completing the trial. Recruitment ended when the required sample of 516 patients had been enrolled. One patient withdrew consent before the study drug was administered and no data were collected for this patient; this patient was excluded from all analyses. Four patients died before the study drug was administered, three before any post-randomisation data on the primary outcome could be collected. These patients have been included in the ITT analysis using data recorded at baseline. Two patients did not receive the study drug for other reasons, but data were collected and they too have been included in the ITT analysis.

Appendix 1 (see *Table 20*) provides a list of the study centres with details of the numbers of patients screened and randomised into each group, along with the number of protocol deviations. There were a total of 100 protocol deviations, all of which were minor (they did not significantly affect patient safety or the scientific value of the trial).

Recruitment and retention

Recruitment lasted for 23 months, from January 2014 to December 2015. There was a temporary halt to recruitment between July and September 2014 when the study drug was recalled and repackaged because of moisture damage to some outer cardboard storage boxes.

The actual recruitment period was shorter than the original target of 2.5 years.

Recruitment rate

The target recruitment rate for the study was one to two patients per month per centre, with a target recruitment figure of 516.

The accrual of patients during the whole study period is presented in *Appendix 1* (see *Figure 28*).

Baseline data and longitudinal monitoring

Baseline data

Participant characteristics at baseline are shown in *Table 5*, with the number of missing values (if any) shown in *Appendix 1* (see *Table 21*).

Longitudinal measurements

The box plots in *Figures 3–14* show the clinical measures monitored at varying intervals throughout the ICU stay. All group allocations are on an ITT basis.

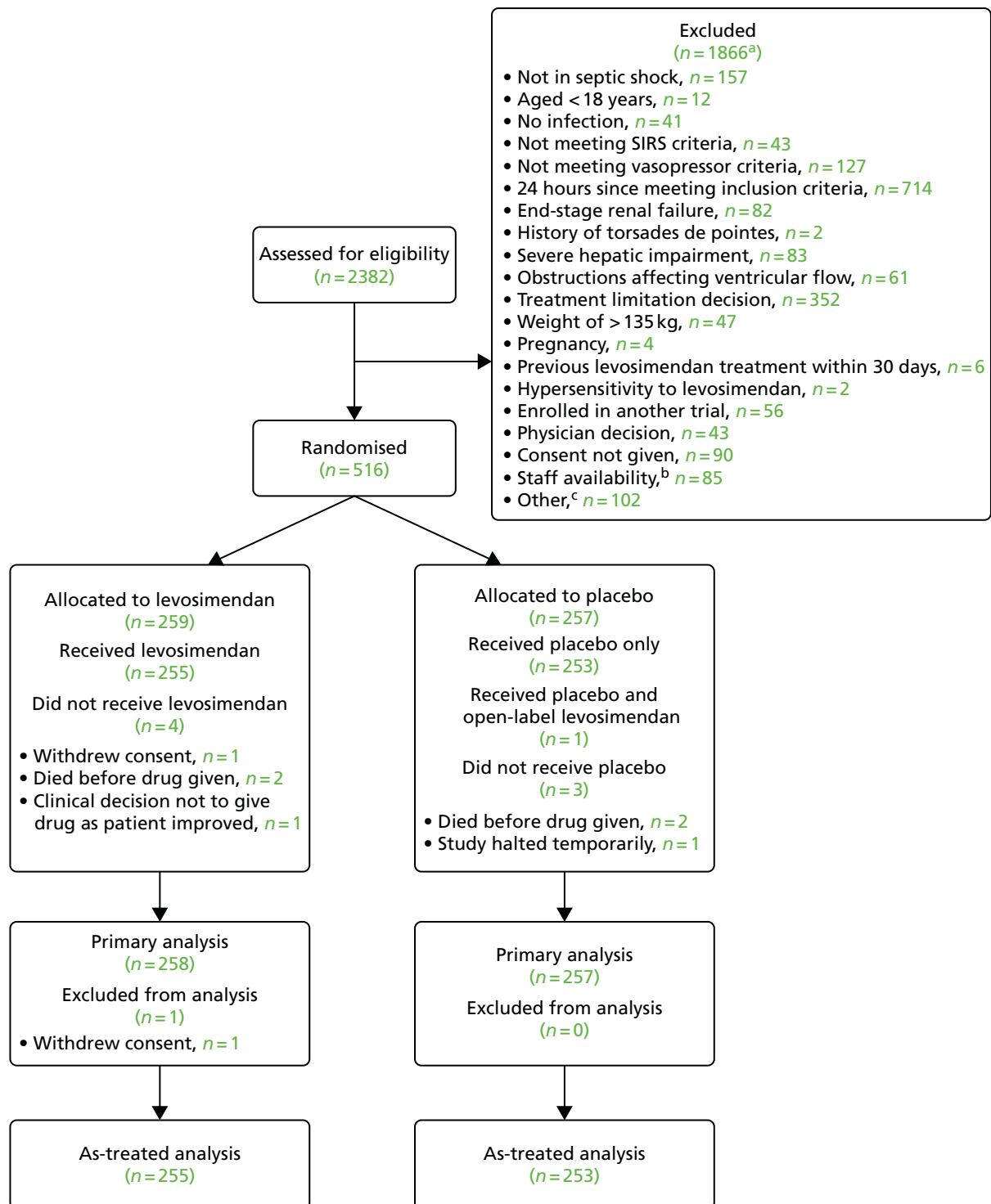


FIGURE 2 Participant flow in the LeopARDS trial: Consolidated Standards of Reporting Trials (CONSORT) flow diagram. a, Patients may have more than one reason for exclusion. b, Patients met all criteria, but could not be randomised because of staff availability. c, Other known reasons for exclusion were unavailability of study drug, patient mental health concerns and language barriers; reason not known for 78 patients.

TABLE 5 Baseline characteristics^a

Characteristic	Treatment group		
	Levosimendan	Placebo	Total
Age (years)	67 (58, 75)	69 (58, 77)	68 (58, 76)
Sex (male)	145 (56)	144 (56)	289 (56)
Weight (kg)	76 (65, 90)	80 (68, 91)	79 (66, 90)
BMI (kg/m ²)	27 (23, 30)	28 (24, 32)	27 (23, 31)
Ethnicity			
Asian	11 (4)	10 (4)	21 (4)
Black	4 (2)	6 (2)	10 (2)
Caucasian	240 (93)	240 (93)	480 (93)
Other	3 (1)	1 (0)	4 (1)
Recent surgical history	94 (36)	95 (37)	189 (37)
APACHE II score	25 (21, 31)	25 (21, 30)	25 (21, 30)
Pre-existing conditions			
Ischaemic heart disease	46 (18)	31 (12)	77 (15)
Congestive heart failure	1 (0)	4 (2)	5 (1)
Cardiac failure	23 (9)	26 (10)	49 (10)
Severe COPD	16 (6)	11 (4)	27 (5)
Chronic renal failure	19 (7)	18 (7)	37 (7)
Cirrhosis	4 (2)	6 (2)	10 (2)
Immunocompromised	23 (9)	24 (9)	47 (9)
Diabetes	59 (23)	51 (20)	110 (21)
Beta-blockers normally taken	54 (21)	45 (18)	99 (19)
Organ failure ^b			
Respiratory	99 (39)	101 (39)	200 (39)
Renal	77 (30)	74 (29)	151 (29)
Liver	6 (2)	8 (3)	14 (3)
Haematological	16 (6)	13 (5)	29 (6)
Neurological	117 (52)	111 (52)	228 (52)
Source of infection			
Lung	98 (38)	103 (40)	201 (39)
Abdomen	95 (37)	96 (37)	191 (37)
Urine	12 (5)	17 (7)	29 (6)
Primary bacteraemia	7 (3)	3 (1)	10 (2)
Neurological	4 (2)	1 (0)	5 (1)
Soft tissue or line	16 (6)	10 (4)	26 (5)
Other	25 (10)	27 (11)	52 (10)

continued

TABLE 5 Baseline characteristics^a (continued)

Characteristic	Treatment group		
	Levosimendan	Placebo	Total
Mechanical ventilation	207 (80)	210 (82)	417 (81)
RRT	44 (17)	45 (18)	89 (17)
Moderate or severe ARDS	72 (28)	59 (23)	131 (25)
Heart rhythm			
Sinus rhythm	201 (78)	218 (85)	419 (82)
Atrial fibrillation	32 (12)	21 (8)	53 (10)
Paced	3 (1)	2 (1)	5 (1)
Other irregular rhythm	22 (9)	14 (5)	36 (7)
Physiological variables			
MAP (mmHg)	74 (68, 80)	73 (67, 79)	74 (68, 79)
Heart rate (beats/minute)	97 (82, 111)	94 (80, 110)	95 (80, 110)
Central venous pressure (mmHg)	11 (9, 15)	12 (8, 16)	11 (8, 15)
Cardiac index (l/minute/m ²) ^c	2.7 (2.2, 3.7)	3.3 (2.2, 4.0)	3 (2.2, 3.8)
SaO ₂ (%)	97 (95, 98)	97 (95, 98)	97 (95, 98)
ScvO ₂ (%)	75 (69, 81)	76 (70, 81)	76 (69, 81)
Lactate (mmol/l)	2.2 (1.4, 3.5)	2.3 (1.5, 3.9)	2.3 (1.4, 3.6)
PaO ₂ /FIO ₂ (kPa)	29 (20, 39)	29 (20, 39)	29 (20, 39)
Creatinine (µmol/l)	140 (89, 216)	137 (93, 208)	138 (91, 213)
Bilirubin (µmol/l)	14 (8, 25)	15 (9, 27)	14 (8, 26)
Haemoglobin (g/l)	108 (94, 123)	108 (93, 125)	108 (94, 124)
Platelets (×10 ⁹ /l)	212 (134, 299)	216 (144, 308)	215 (140, 307)
GCS score	9 (3, 15)	8 (3, 15)	9 (3, 15)
Time from shock to randomisation (hours) ^d	16 (10, 21)	15 (10, 20)	16 (10, 21)
Vasoactive drug dosage at randomisation			
Noradrenaline (µg/kg/minute)	0.29 (0.16, 0.52), n = 255	0.27 (0.15, 0.44), n = 253	0.28 (0.16, 0.47), n = 508
Adrenaline (µg/kg/minute)	0.14 (0.07, 0.28), n = 21	0.13 (0.08, 0.38), n = 21	0.14 (0.07, 0.3), n = 42
Vasopressin (units/minute)	0.03 (0.02, 0.04), n = 33	0.03 (0.02, 0.04), n = 37	0.03 (0.02, 0.04), n = 70
Dobutamine (µg/kg/minute)	5.7 (3.5, 8.8), n = 18	5 (4.4, 6.2), n = 22	5.2 (4.4, 6.5), n = 40

ARDS, acute respiratory distress syndrome; BMI, body mass index; COPD, chronic obstructive pulmonary disease; GCS, Glasgow Coma Scale.

a Data are median (lower quartile, upper quartile values) for continuous variables and *n* (%) for dichotomous and categorical variables.

b Renal failure is defined as having AKI stage 3 (urine output criteria omitted as data unavailable); other organ failures defined as having a SOFA score of ≥ 3.

c Measured in 84 levosimendan patients and 73 placebo patients. Rates of other missing values are shown in Table 21.

d Time from onset of shock (starting vasopressors) to randomisation.

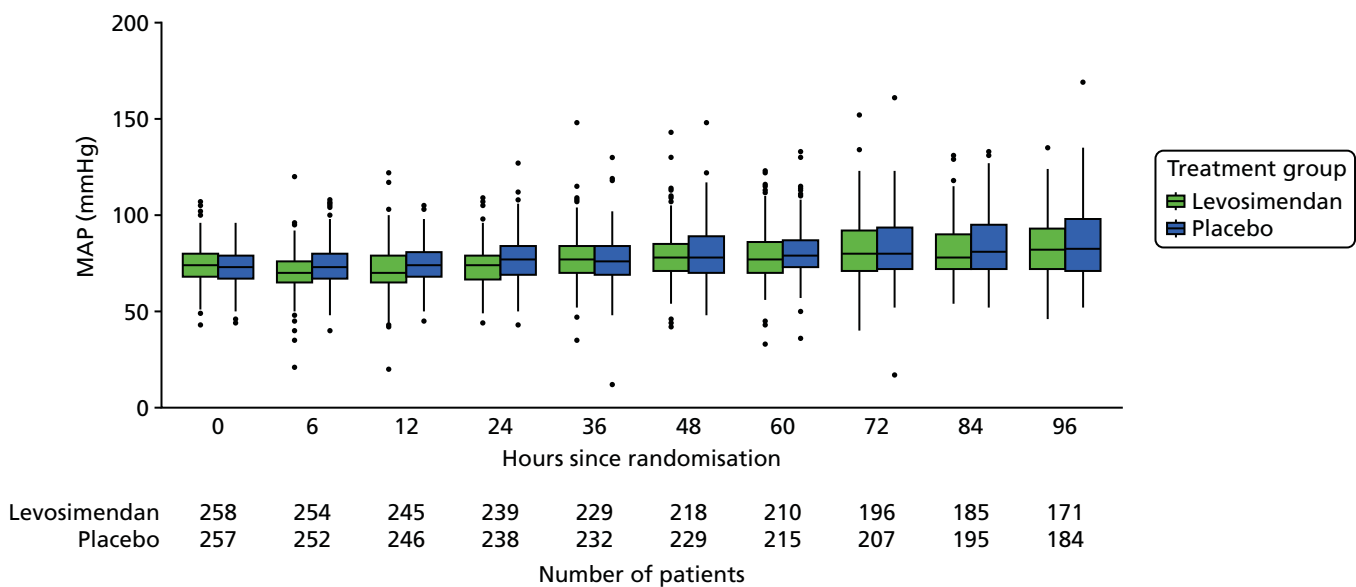


FIGURE 3 Box plot for MAP, by treatment group. Line = median; box = IQR; whiskers = extremes of the data ($1.5 \times$ the IQR); circles = very extreme outliers. The data shown include all patients still alive and still in the ICU for each time point since randomisation.

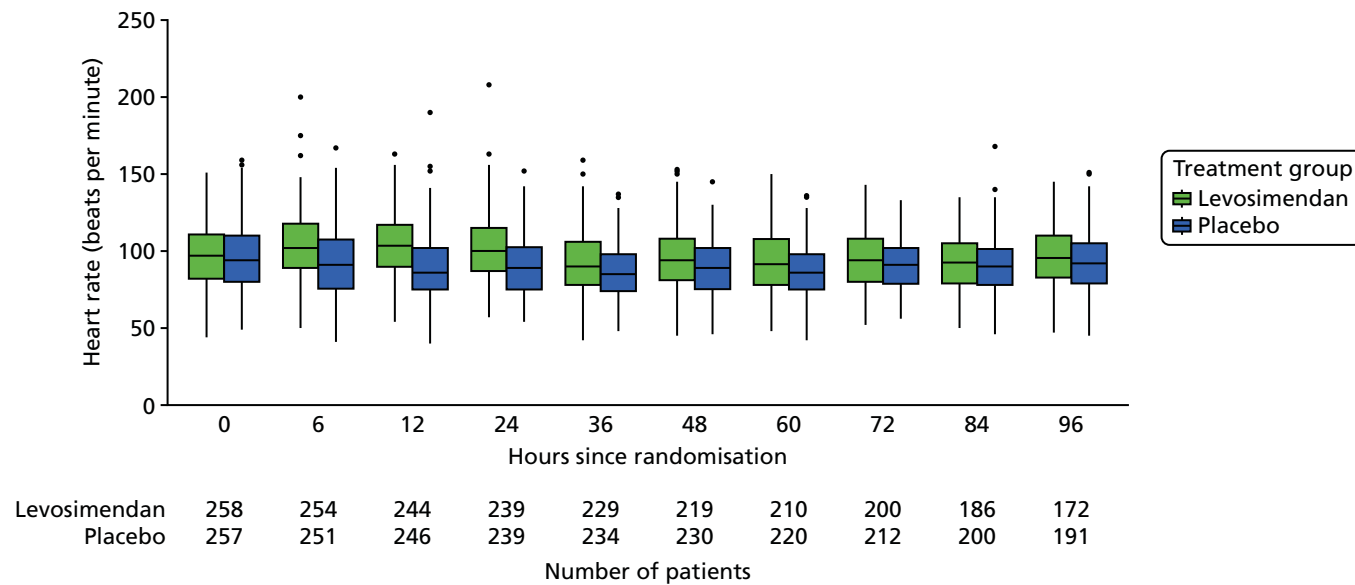


FIGURE 4 Box plot for heart rate, by treatment group. Line = median; box = IQR; whiskers = extremes of the data (1.5 × the IQR); circles = very extreme outliers. The data shown include all patients still alive and still in the ICU for each time point since randomisation.

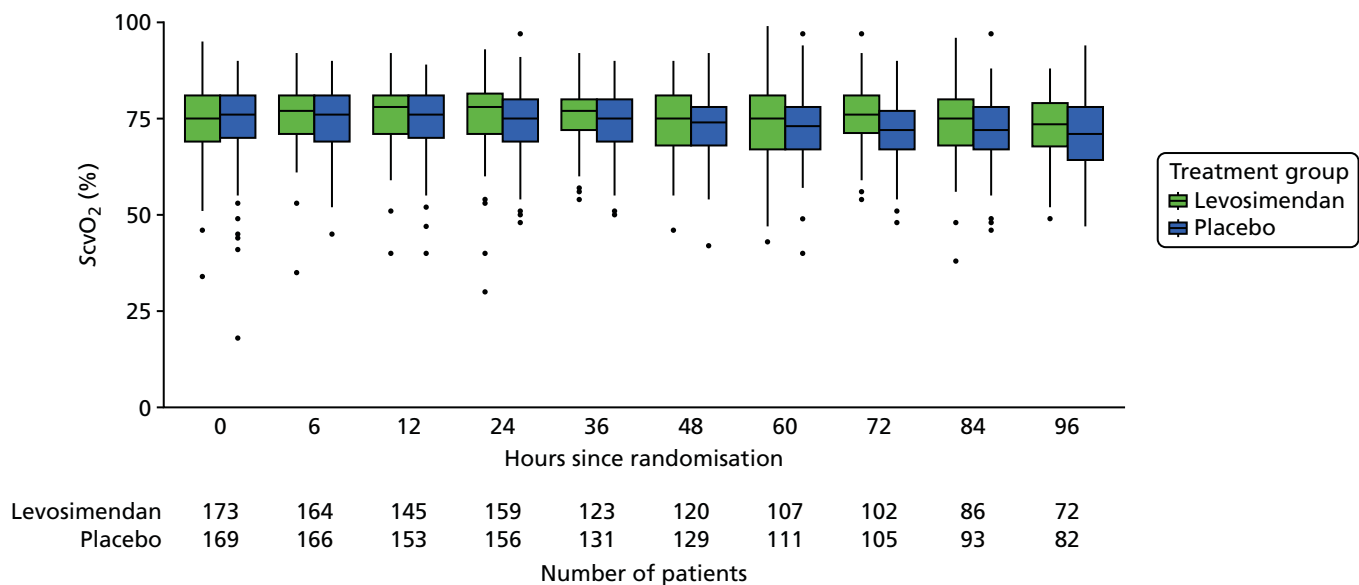


FIGURE 5 Box plot for $ScvO_2$, by treatment group. Line = median; box = IQR; whiskers = extremes of the data ($1.5 \times$ the IQR); circles = very extreme outliers. The data shown include all patients still alive and still in the ICU for each time point since randomisation.

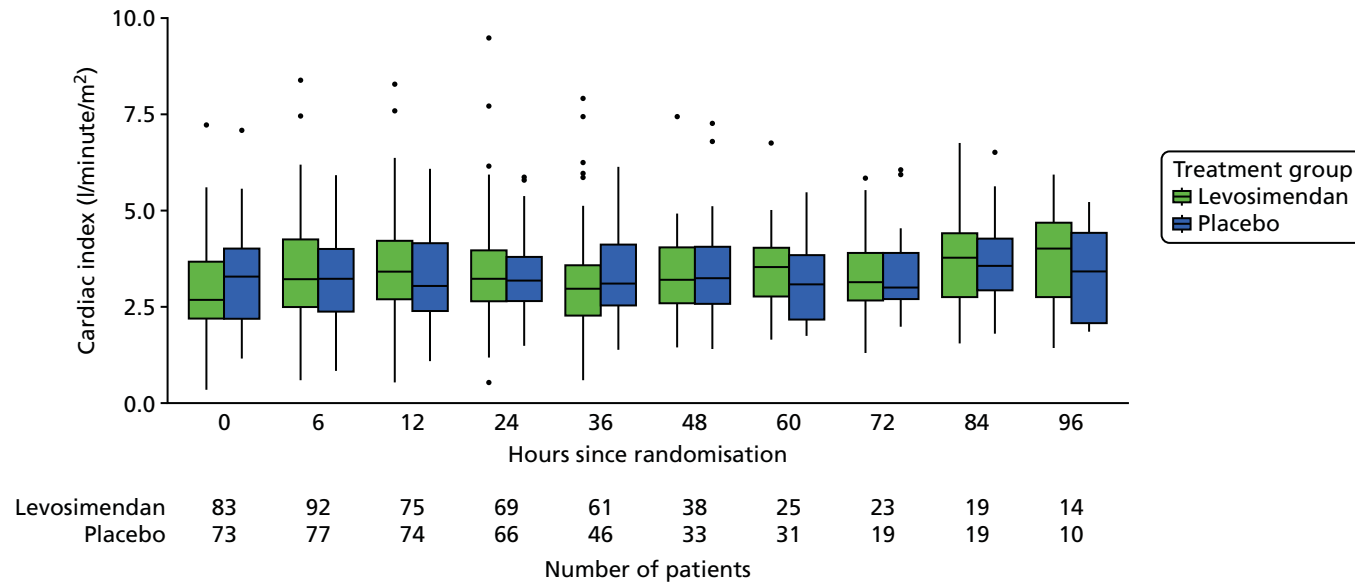


FIGURE 6 Box plot for cardiac index, by treatment group. Line = median; box = IQR; whiskers = extremes of the data (1.5 × the IQR); circles = very extreme outliers. The data shown include all patients still alive and still in the ICU for each time point since randomisation.

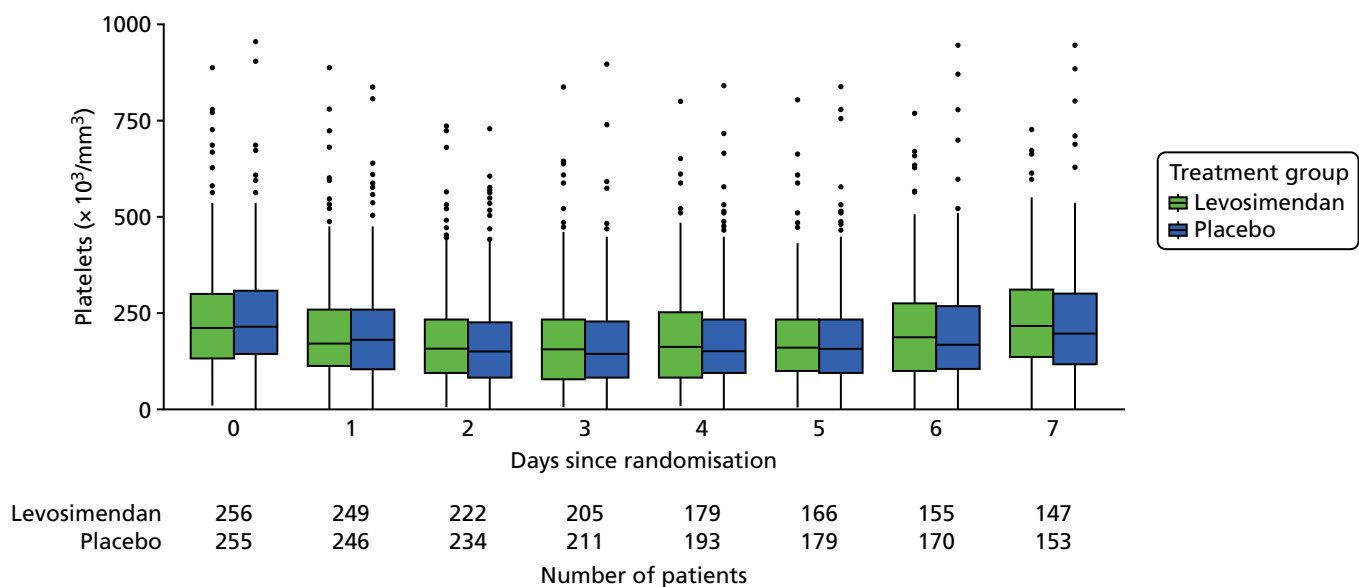


FIGURE 7 Box plot for platelet count, by treatment group. Line = median; box = IQR; whiskers = extremes of the data ($1.5 \times$ the IQR); circles = very extreme outliers. The data shown include all patients still alive and still in the ICU for each time point since randomisation.

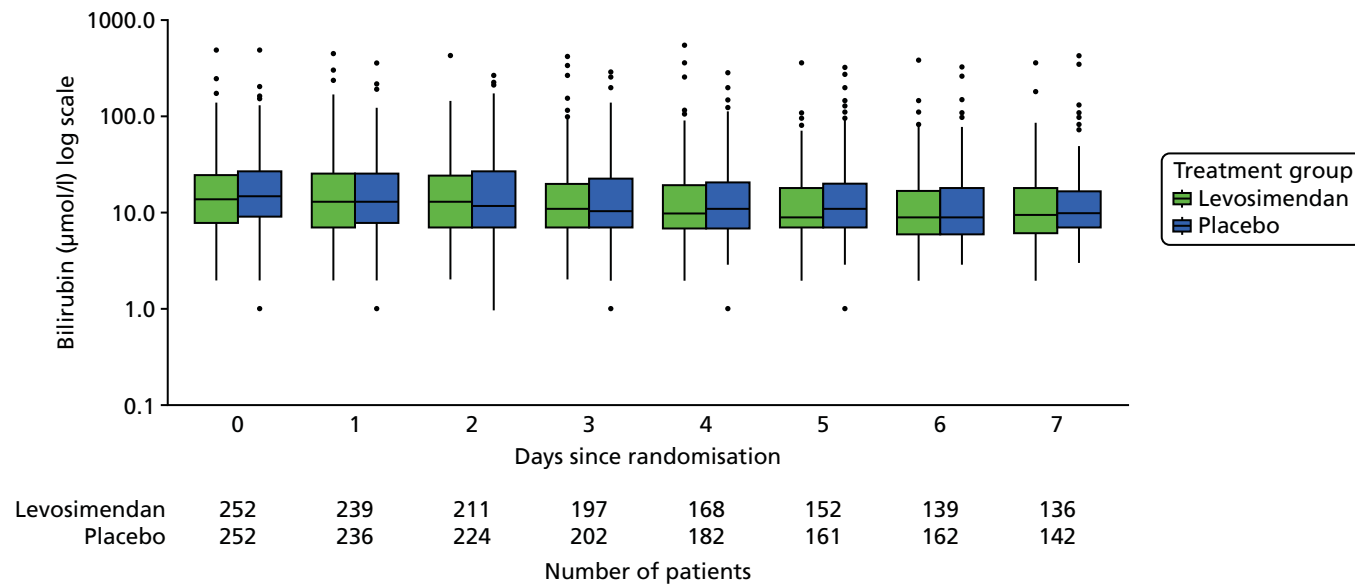


FIGURE 8 Box plot for bilirubin level, by treatment group. Line = median; box = IQR; whiskers = extremes of the data ($1.5 \times$ the IQR); circles = very extreme outliers. The data shown include all patients still alive and still in the ICU for each time point since randomisation.

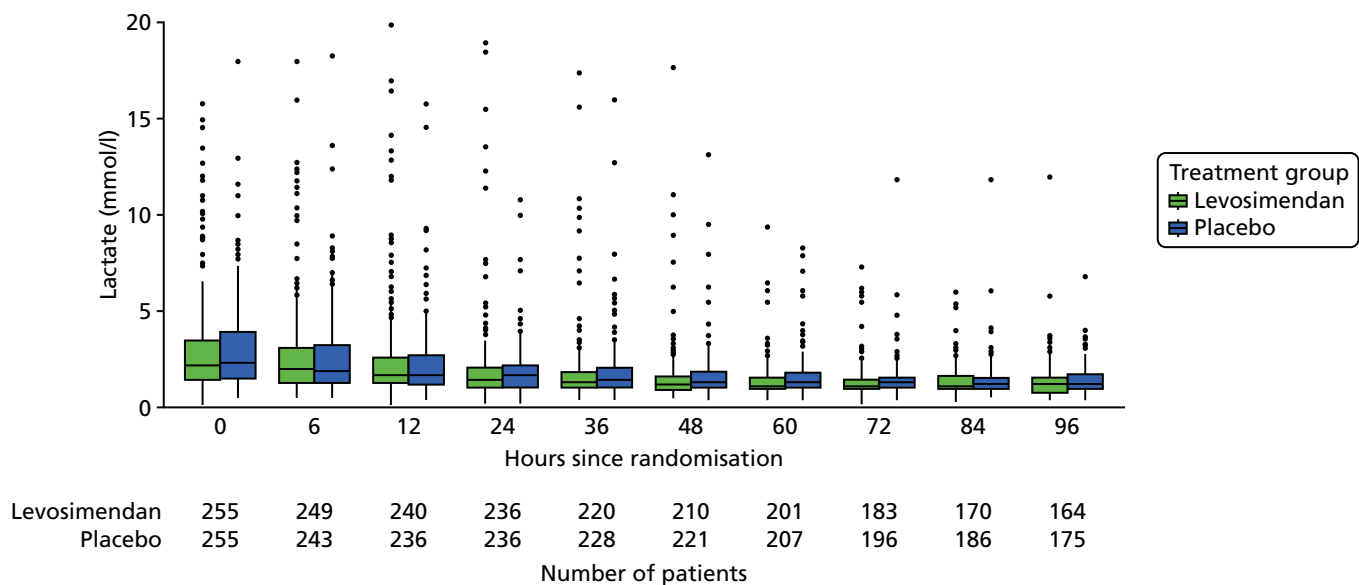


FIGURE 9 Box plot for lactate level, by treatment group. Line = median; box = IQR; whiskers = extremes of the data ($1.5 \times$ the IQR); circles = very extreme outliers. The data shown include all patients still alive and still in the ICU for each time point since randomisation.

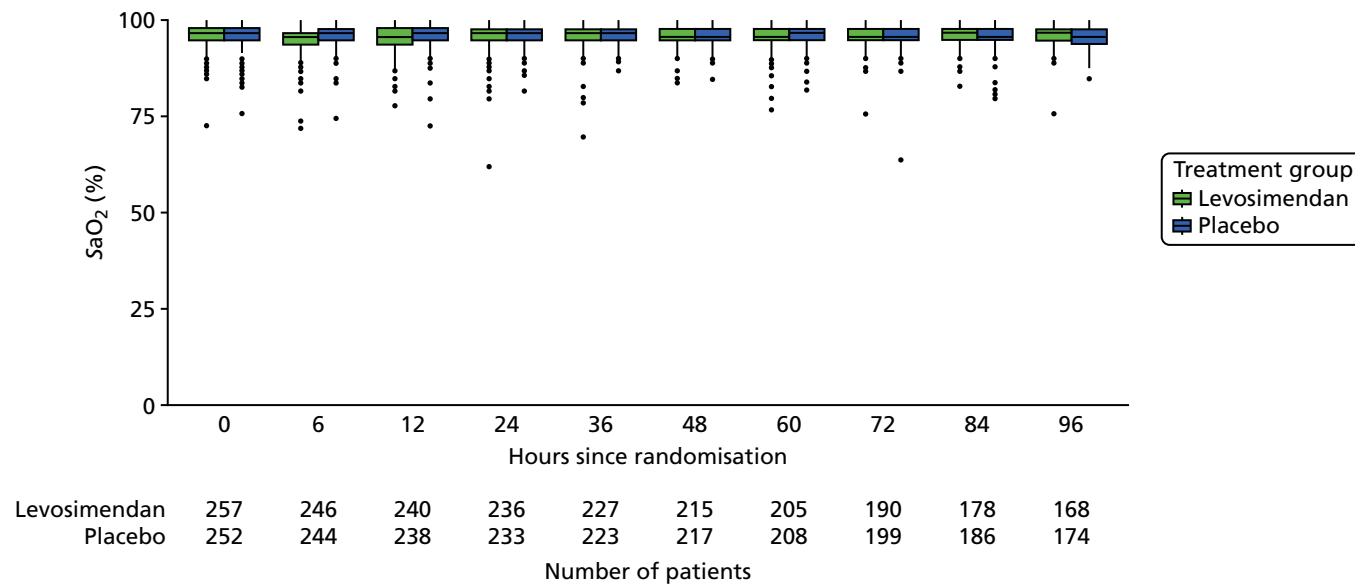


FIGURE 10 Box plot for SaO₂, by treatment group. Line = median; box = IQR; whiskers = extremes of the data (1.5 × the IQR); circles = very extreme outliers. The data shown include all patients still alive and still in the ICU for each time point since randomisation.

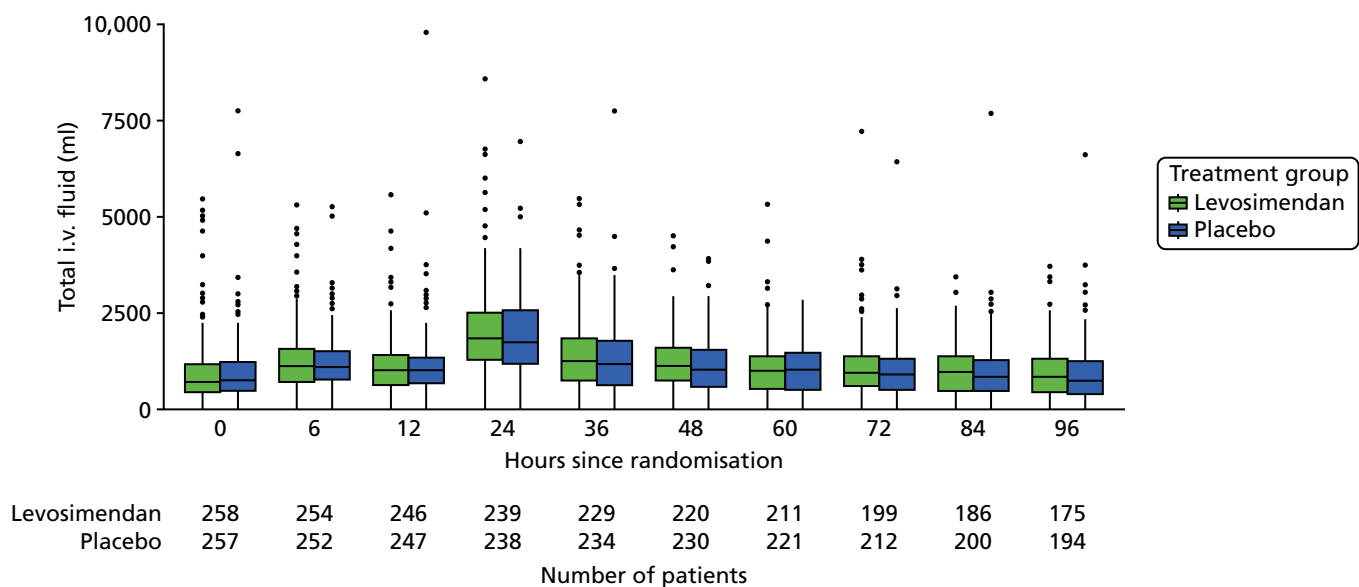


FIGURE 11 Box plot for total intravenous (i.v.) fluid, by treatment group. Line = median; box = IQR; whiskers = extremes of the data ($1.5 \times$ the IQR); circles = very extreme outliers. The data shown include all patients still alive and still in the ICU for each time point since randomisation.

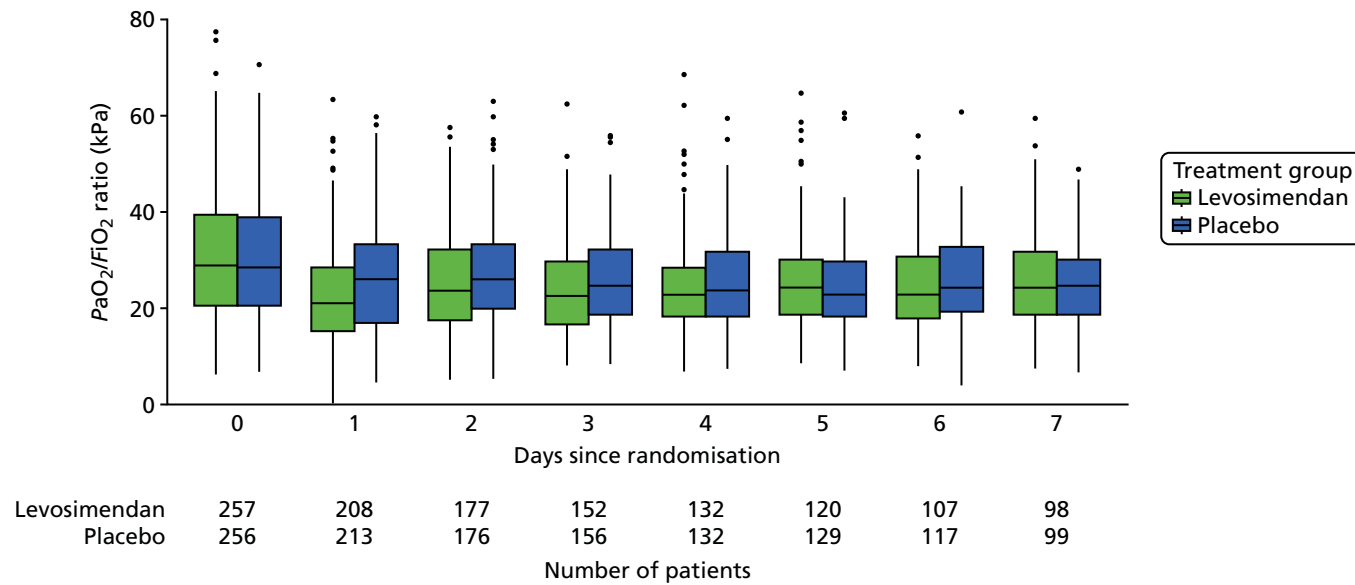


FIGURE 12 Box plot for PaO_2/FiO_2 ratio, by treatment group. Line = median; box = IQR; whiskers = extremes of the data ($1.5 \times$ the IQR); circles = very extreme outliers. The data shown include all patients still alive and still in the ICU for each time point since randomisation.

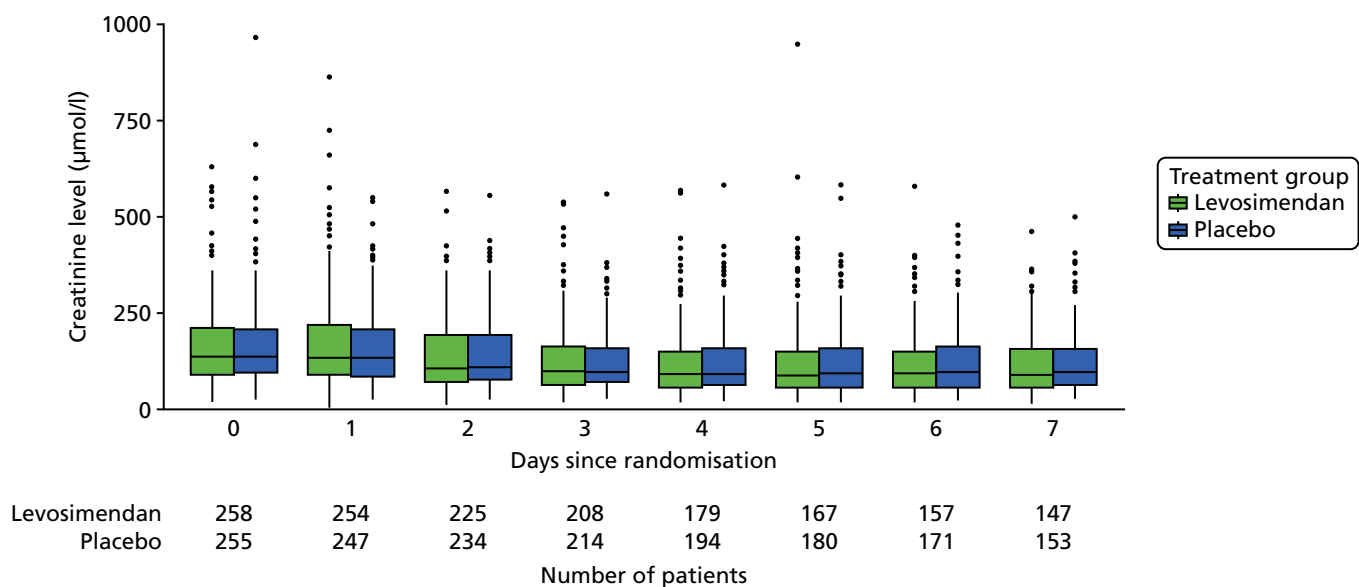


FIGURE 13 Box plot for creatinine level, by treatment group. Line = median; box = IQR; whiskers = extremes of the data ($1.5 \times$ the IQR); circles = very extreme outliers. The data shown include all patients still alive and still in the ICU for each time point since randomisation.

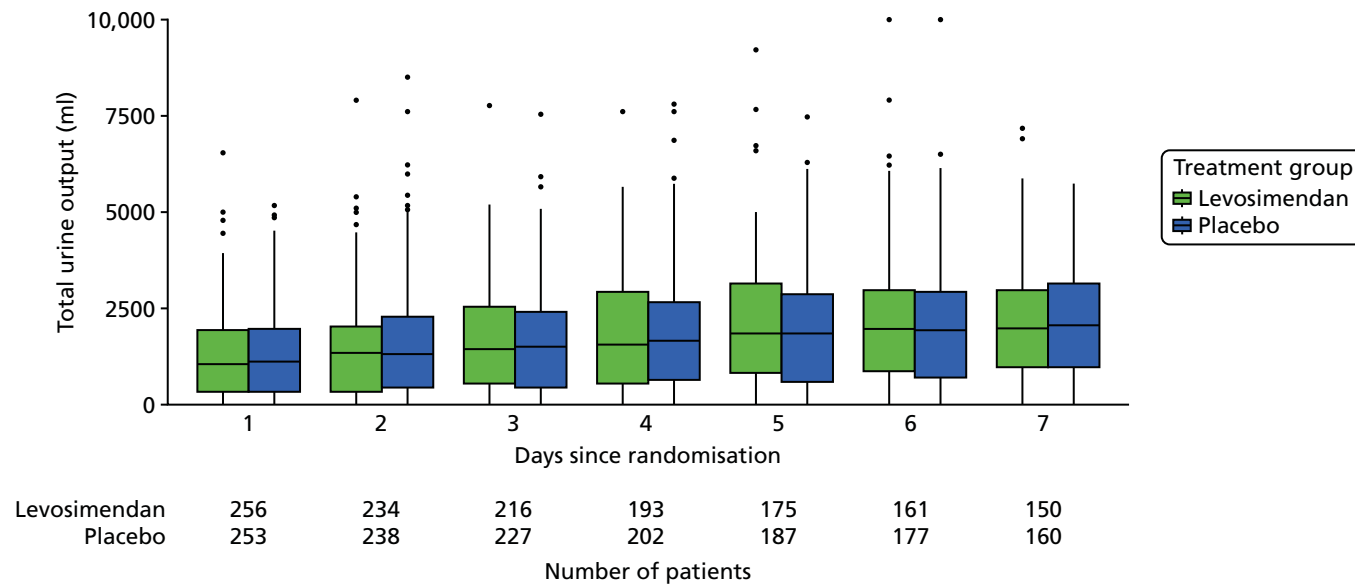


FIGURE 14 Box plot for total urine output, by treatment group. Line = median; box = IQR; whiskers = extremes of the data ($1.5 \times$ the IQR); circles = very extreme outliers. The data shown include all patients still alive and still in the ICU for each time point since randomisation.

Study drug infusion

Table 6 shows the numbers of patients receiving the study drug by dose at 6, 12 and 24 hours in the levosimendan and placebo groups.

Other vasoactive drugs

Tables 7 and 8 show the numbers of patients receiving noradrenaline and dobutamine, respectively, by group and the median dose received at each time point.

Safety data

Table 9 shows the numbers of AEs by SAE classification and their relationship to the study medication.

TABLE 6 Numbers of patients receiving the study drug, by dose at 6, 12 and 24 hours

Dose ($\mu\text{g}/\text{kg}/\text{minute}$)	Treatment group, <i>n</i> (%)		Total, <i>n</i> (%)
	Levosimendan	Placebo	
6 hours			
0	20 (7.8)	8 (3.1)	28 (5.4)
0.05	36 (14.0)	11 (4.3)	47 (9.1)
0.1	43 (16.7)	22 (8.6)	65 (12.6)
0.2	159 (61.6)	216 (84.0)	375 (72.8)
Total	258 (100)	257 (100)	515 (100)
12 hours			
0	28 (11.3)	7 (2.8)	35 (7.0)
0.05	32 (12.9)	9 (3.6)	41 (8.2)
0.1	41 (16.5)	22 (8.8)	63 (12.7)
0.2	147 (59.3)	212 (84.8)	359 (72.1)
Total	248 (100)	250 (100)	498 (100)
24 hours			
0	33 (13.5)	19 (7.7)	52 (10.6)
0.05	26 (10.7)	6 (2.4)	32 (6.5)
0.1	38 (15.6)	8 (3.2)	46 (9.3)
0.2	147 (60.2)	215 (86.7)	362 (73.6)
Total	244 (100)	248 (100)	492 (100)

TABLE 7 Numbers of patients receiving noradrenaline, by treatment group and the median dose ($\mu\text{g}/\text{kg}/\text{minute}$) received at each follow-up point from randomisation

Follow-up (hours)	Treatment group								
	Levosimendan			Placebo			Total		
	n (%)	Median dose	LQ, UQ	n (%)	Median dose	LQ, UQ	n (%)	Median dose	LQ, UQ
6	246 (95.3)	0.32	0.17, 0.53	240 (93.4)	0.24	0.13, 0.44	486 (94.4)	0.29	0.15, 0.50
12	238 (96.0)	0.30	0.16, 0.54	232 (92.8)	0.20	0.10, 0.39	470 (94.4)	0.26	0.12, 0.47
24	210 (86.1)	0.28	0.14, 0.46	195 (78.6)	0.18	0.07, 0.33	405 (82.3)	0.22	0.11, 0.40
36	185 (80.8)	0.18	0.09, 0.32	167 (71.4)	0.15	0.07, 0.29	352 (76.0)	0.16	0.08, 0.31
48	145 (63.9)	0.13	0.07, 0.26	124 (53.2)	0.12	0.07, 0.24	269 (58.5)	0.13	0.07, 0.25
60	121 (57.3)	0.11	0.05, 0.25	107 (48.9)	0.12	0.06, 0.20	228 (53.0)	0.12	0.05, 0.24
72	92 (44.4)	0.11	0.06, 0.21	78 (36.8)	0.09	0.05, 0.16	170 (40.6)	0.10	0.06, 0.20
84	76 (40.6)	0.10	0.06, 0.22	66 (33.7)	0.09	0.05, 0.17	142 (37.1)	0.10	0.05, 0.21
96	58 (32.0)	0.11	0.06, 0.24	61 (31.8)	0.08	0.03, 0.17	119 (31.9)	0.10	0.04, 0.20

LQ, lower quartile value; UQ, upper quartile value.

TABLE 8 Numbers of patients receiving dobutamine, by treatment group and the median dose ($\mu\text{g}/\text{kg}/\text{minute}$) received at each follow-up point from randomisation

Follow-up (hours)	Treatment group								
	Levosimendan			Placebo			Total		
	n (%)	Median dose	LQ, UQ	n (%)	Median dose	LQ, UQ	n (%)	Median dose	LQ, UQ
6	13 (5.0)	5.00	4.00, 9.10	24 (9.3)	5.00	4.40, 5.65	37 (7.2)	5.00	4.40, 6.40
12	10 (4.0)	6.10	5.00, 8.80	23 (9.2)	4.40	3.50, 5.25	33 (6.6)	4.90	3.70, 6.40
24	10 (4.1)	5.65	2.70, 7.00	22 (8.9)	4.55	3.40, 5.50	32 (6.5)	4.75	3.30, 6.32
36	7 (3.1)	5.50	1.95, 7.25	20 (8.5)	4.55	3.70, 6.40	27 (5.8)	4.60	2.70, 6.85
48	4 (1.8)	5.95	4.45, 7.93	18 (7.7)	4.20	3.08, 4.90	22 (4.8)	4.20	3.08, 5.57
60	1 (0.5)	1.30	1.30, 1.30	18 (8.2)	3.70	2.05, 6.05	19 (4.4)	3.30	1.65, 5.80
72	2 (1.0)	7.20	4.55, 9.85	16 (7.5)	3.90	1.73, 5.55	18 (4.3)	3.90	1.83, 6.05
84	1 (0.5)	1.90	1.90, 1.90	11 (5.6)	4.60	1.65, 6.60	12 (3.1)	4.40	1.73, 6.45
96	3 (1.7)	2.30	2.10, 3.75	10 (5.2)	4.40	2.00, 6.05	13 (3.5)	4.20	1.90, 5.30

LQ, lower quartile value; UQ, upper quartile value.

TABLE 9 Numbers of AEs by SAE classification and their relationship to the study medication: ITT analysis

Classification	Relationship ^a	Treatment group			
		Number of AEs ^b		Number of subjects ^c	
		Levosimendan	Placebo	Levosimendan	Placebo
Not serious	Definite	1	0	1	0
	Not assessable	0	1	0	1
	Not related	26	13	7	4
	Possible	24	16	19	13
	Probable	6	0	5	0
	Unlikely	16	16	8	10
Serious	Definite	0	0	0	0
	Not assessable	0	0	0	0
	Not related	9	11	8	10
	Possible	15	3	12	1
	Probable	1	0	1	0
	Unlikely	13	11	11	11
Total	All	111	71	72	50

a Relationship to study medication.

b A subject may have more than one AE.

c A subject is shown only once, using their AE with the most serious classification and highest level of causality.

Multiple entries for the same AE are counted only once (according to the most serious classification and highest level of causality).

Primary outcome analysis

Exploratory analysis

Figure 15 provides a box plot of the total SOFA score by group and day since randomisation.

Missing data

Appendix 1 (see Table 22) shows the level of missingness across subjects and across daily scores by treatment group. In total, 66% of patients had at least one total SOFA score missing and hence an incomplete set of scores on which to base the primary outcome of mean total SOFA score. Although this is high, each patient should have five component scores recorded for each day in the ICU and missing only one of these would result in the patient having at least one missing total SOFA score. Of the 26,775 scores expected (one for each of the five components for each day that each patient was in the ICU), 1773 (6.2%) were missing, only slightly higher than the 4% anticipated. In the LeoPARDS trial, there was an expectation that scores might not be recorded for 1 or 2 days when there had been no change or when the patient was getting better and approaching discharge. There were 60 instances (across all components) when ≥ 3 consecutive days were missing that did not occur at the end of the ICU stay.

For the main analysis we used a version of LOCF, as described in Chapter 3, *Missing data*, with a Bayesian sensitivity analysis to check where the results were sensitive to the underestimation of uncertainty in the LOCF approach.

Main analysis of the primary outcome

Table 10 shows the main analysis of the primary outcome (mean total SOFA score averaged over all days that a patient was in the ICU), along with each of the SOFA components separately. Three subjects died soon after randomisation and before any data could be collected; they were assigned baseline values in

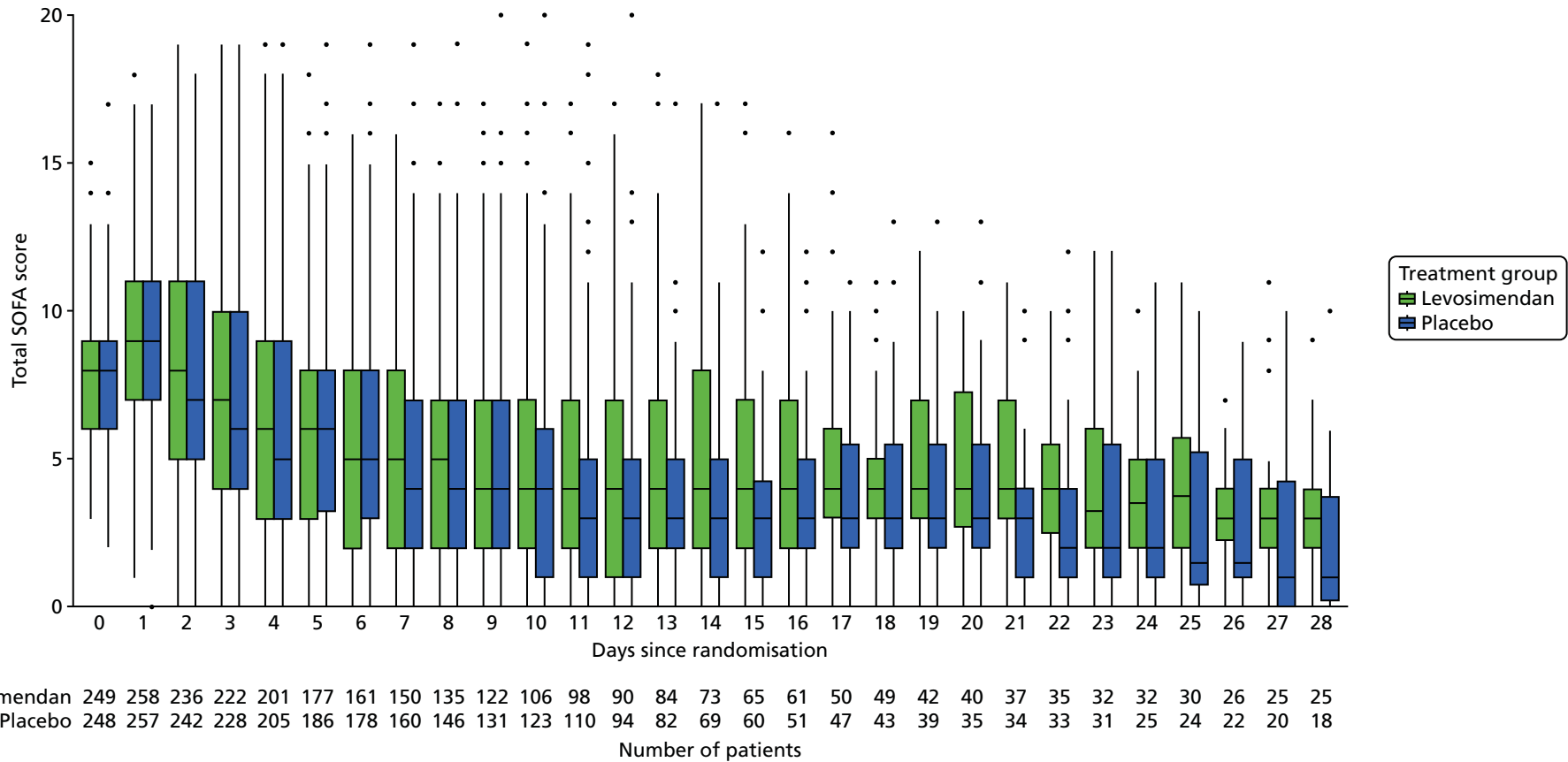


FIGURE 15 Box plot of total SOFA scores, by day and treatment group.

TABLE 10 Mean total SOFA score between randomisation and ICU discharge: ITT analysis

SOFA component	Treatment group						Absolute difference (levosimendan – placebo)	
	Levosimendan			Placebo			Mean (95% CI) ^a	Median (95% CI) ^b
	Mean	SD	Median (LQ, UQ)	Mean	SD	Median (LQ, UQ)		
Respiration	1.70	1.18	1.6 (0.76, 2.68)	1.56	1.15	1.5 (0.50, 2.36)	0.14 (–0.06 to 0.34)	0.10 (–0.20 to 0.54)
Coagulation	0.75	1.05	0.14 (0.00, 1.19)	0.75	1.02	0.3 (0.00, 1.08)	0.00 (–0.18 to 0.17)	–0.16 (–0.36 to 0.11)
Liver	0.51	0.84	0.00 (0.00, 0.74)	0.45	0.77	0.00 (0.00, 0.64)	0.06 (–0.08 to 0.19)	0.00 (0.00 to 0.05)
Cardiovascular	2.27	1.20	2.00 (1.22, 3.52)	2.02	1.20	1.67 (1.00, 3.04)	0.25 (0.04 to 0.46)	0.33 (0.07 to 0.62)
Renal	1.46	1.49	1.00 (0.00, 2.88)	1.28	1.38	0.79 (0.00, 2.25)	0.18 (–0.07 to 0.42)	0.21 (–0.29 to 0.63)
Total	6.68	3.96	5.63 (3.75, 9.00)	6.06	3.89	5.13 (3.33, 7.86)	0.61 (–0.07 to 1.29)	0.50 (–0.28 to 1.25)

LQ, lower quartile value; UQ, upper quartile value.
a Calculated using bootstrapping.
b Presented on the square root scale as there is no suitable back-transform to an interpretable scale.

line with the LOCF imputation. A further two subjects who both had only one daily measurement taken on the ICU before they died were missing the liver component for this day, with no baseline measurement; these patients were assumed to have a normal SOFA score based on our assumptions about missing data and with the majority of these scores being normal. *Appendix 1* (see *Table 23*) shows the corresponding 'as-treated' analysis, excluding seven participants who did not receive the study drug and one placebo participant who received open-label levosimendan.

Sensitivity analysis of the primary outcome

Table 11 shows the results of the sensitivity analysis of the primary outcome.

TABLE 11 Regression analysis of the mean total SOFA score: estimated parameters with bootstrapped 95% CIs

Variable	Model	
	1 ^a	2 ^a
Intercept	0.63 (–1.16 to 2.42)	1.19 (0.84 to 1.54)
Treatment difference ^b	0.59 (–0.02 to 1.20)	0.13 (0.02 to 0.25)
Age (years)	–0.01 (–0.03 to 0.01)	0.00 (–0.01 to 0.00)
APACHE II score	0.24 (0.20 to 0.28)	0.05 (0.04 to 0.06)

a Response is the mean total SOFA score, untransformed for model 1 and with square root transformation for model 2.
b Absolute difference for levosimendan – placebo.

Subgroup analysis of the primary outcome

Figure 16 shows the results of the subgroup analysis of the primary outcome.

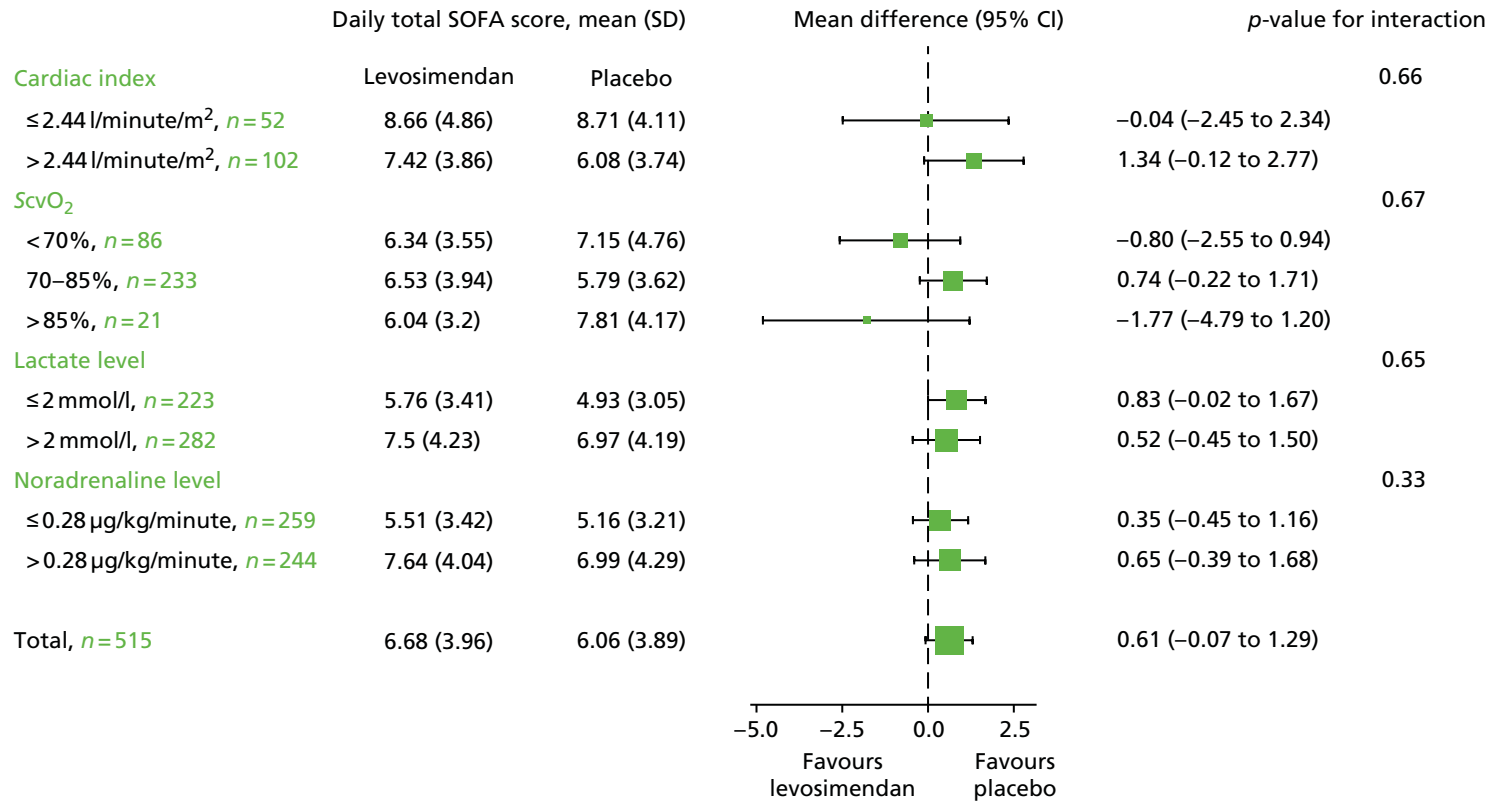


FIGURE 16 Forest plot showing the difference in mean SOFA score, by predefined subgroup.

Secondary outcomes

Time to extubation

Participants in the levosimendan group were less likely than those in the placebo group to be successfully weaned from mechanical ventilation over 28 days (hazard ratio 0.77, 95% CI 0.60 to 0.97; $p = 0.03$) (Figure 17 and Table 12).

Survival

Figures S4 (see Report Supplementary Material 1) and Figure 18 show the Kaplan–Meier plots for survival to 28 days and 6 months, respectively. Three participants were censored before 6 months. One participant in the placebo group was censored at ICU discharge as post-discharge follow-up was declined. Two participants were censored at 28 days: one participant in the placebo group who had no hospital or primary care data recorded and one participant in the levosimendan group who left the UK.

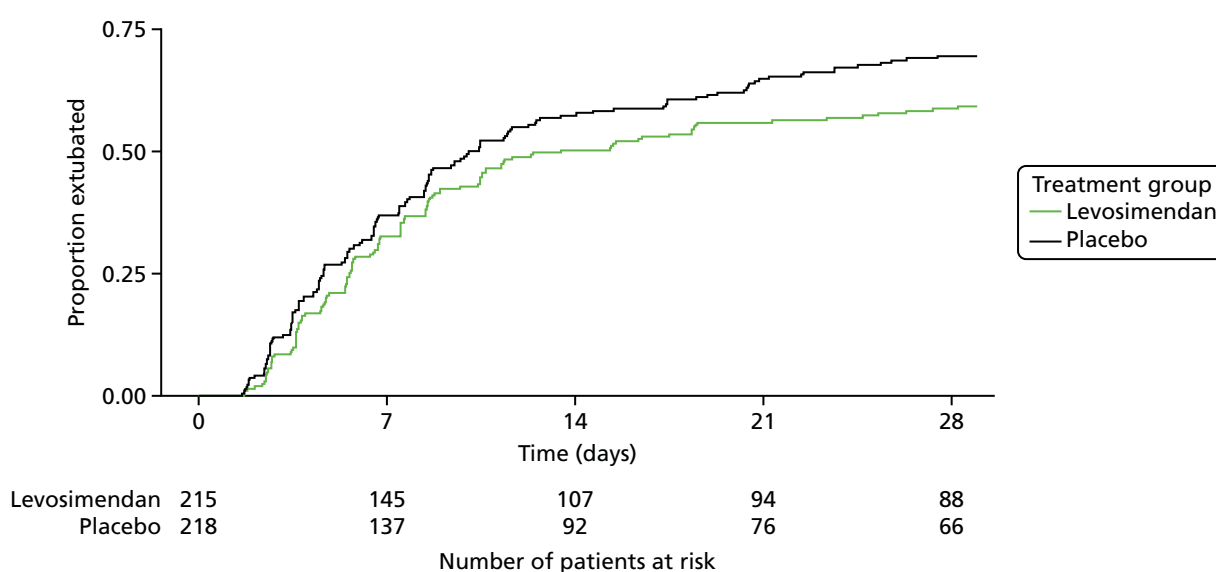


FIGURE 17 Kaplan–Meier plot for time to extubation. The hazard ratio in the levosimendan group compared with the placebo group was 0.77 (95% CI 0.60 to 0.97; $p = 0.03$).

TABLE 12 Cox regression for time to extubation

Variable	Analysis	
	Unadjusted	Adjusted
Treatment difference ^a	0.78 (0.61 to 0.99)	0.77 (0.60 to 0.97)
Age (years)		1.00 (0.99 to 1.01)
APACHE II score		0.94 (0.92 to 0.96)

^a Hazard ratio and 95% CI comparing levosimendan with placebo; adjusted analysis includes random effects for ICU.

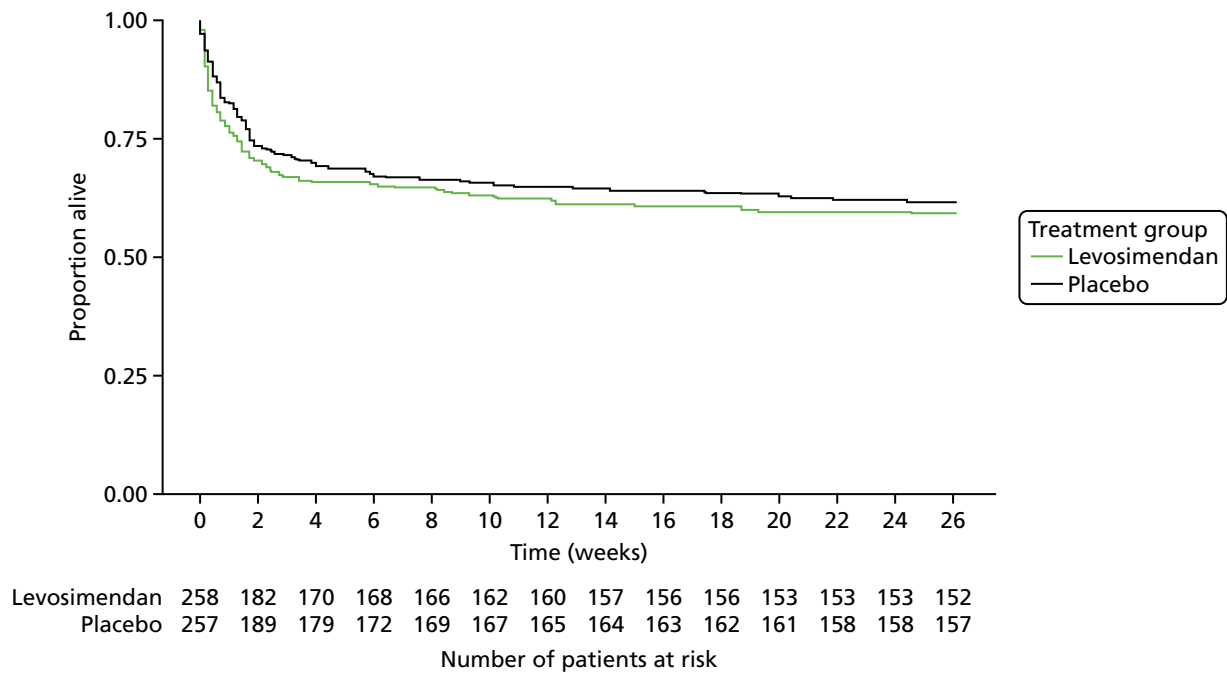


FIGURE 18 Kaplan–Meier plot for survival to 6 months.

Table S14 (see Report Supplementary Material 1) shows 28-day mortality, mortality before discharge from the ICU and from hospital, and 3- and 6-month mortality established from hospital readmission and GP data. Results from the Cox regression, both unadjusted and adjusted for age, APACHE II score and ICU effects, are shown in Tables S15 and S16 (see Report Supplementary Material 1) for 28-day and 6-month survival, respectively.

Figure 19 shows the results of the subgroup analysis of survival at 28 days.

A summary of the clinical outcomes is provided in Table 13.

Biomarker data

Figures S5–S11 (see Report Supplementary Material 1) show the box plots for each biomarker by treatment group and sample day, including baseline samples. All measures are shown on the log scale. The analysis of the change in biomarkers over time between the two treatment groups is reported in Hierarchical regression models for change in biomarkers over time.

Subgroup analysis of mean SOFA scores and 28-day mortality by cardiovascular markers

The mean total SOFA score (both including and excluding the cardiovascular component), the mean SOFA component scores and 28-day mortality were analysed by subgroups of NT-proBNP and troponin values, categorising the study population based on normal and raised values and values below and above the median value in the study population (Figures 20 and 21 and see Report Supplementary Material 1, Tables S25–S32).

Hierarchical regression models for change in biomarkers over time

To assess if levosimendan alters the pro- and anti-inflammatory balance in sepsis the measured inflammatory biomarkers were analysed using Bayesian hierarchical regression models.

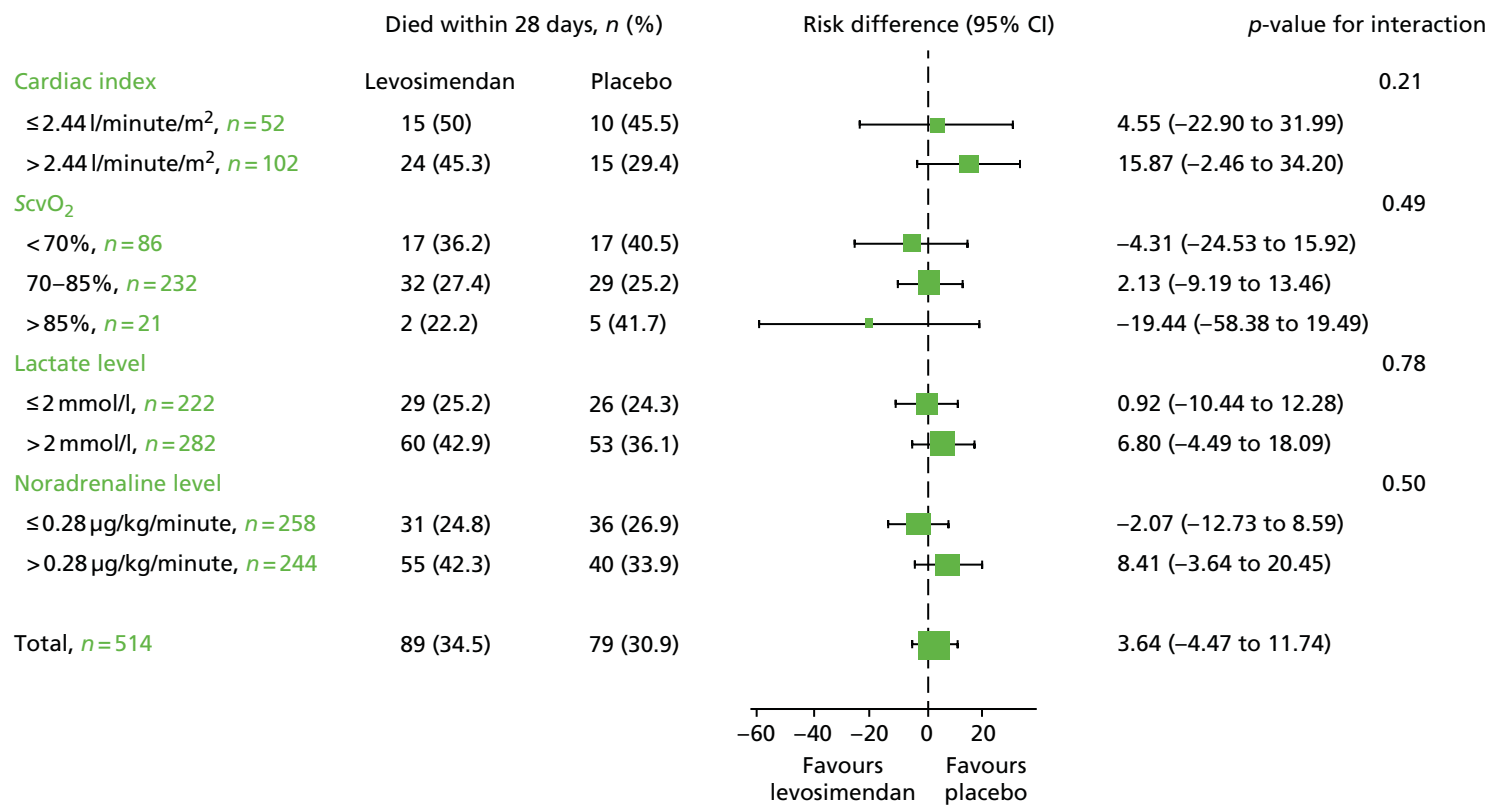


FIGURE 19 Forest plot showing the difference in survival at day 28, by subgroup.

TABLE 13 Summary of clinical outcomes^a

Outcome	Treatment group, n(%)		Absolute difference (95% CI)	p-value ^a
	Levosimendan (N = 258)	Placebo (N = 257)		
Primary outcome				
Mean daily total SOFA score	6.68 (3.96)	6.06 (3.89)	0.61 (−0.07 to 1.29)	0.053
Mean daily respiratory SOFA score	1.70 (1.18)	1.56 (1.15)	0.14 (−0.06 to 0.34)	0.23
Mean daily coagulation SOFA score	0.75 (1.05)	0.75 (1.02)	0.00 (−0.18 to 0.17)	0.55
Mean daily hepatic SOFA score	0.51 (0.84)	0.45 (0.77)	0.06 (−0.08 to 0.19)	0.65
Mean daily cardiovascular SOFA score	2.27 (1.20)	2.02 (1.20)	0.25 (0.04 to 0.46)	0.01
Mean daily renal SOFA score	1.46 (1.49)	1.28 (1.38)	0.18 (−0.07 to 0.42)	0.32
Mean daily SOFA score excluding cardiovascular score	4.41 (3.13)	4.05 (3.07)	0.36 (−0.17 to 0.90)	0.12
Mean daily total SOFA score, sensitivity analysis ^b	7.19 (3.72)	6.78 (3.74)	0.41 (−0.24 to 1.06 ^c)	–
Secondary outcomes				
Mortality				
At 28 days	89 (34.5)	79 (30.9) ^d	3.6 (−4.5 to 11.7)	0.43
At ICU discharge	83 (32.2)	76 (29.6)	2.6 (−5.4 to 10.6)	0.59
At hospital discharge	97 (37.6)	84 (32.8) ^d	4.8 (−3.5 to 13.0)	0.30
Number of catecholamine-free days	22 (0, 26)	23 (0, 26)	−1.0 (−4.5 to 1.0)	0.09
Number of ventilation-free days	16 (0, 25)	19 (0, 25)	−3.0 (−9.5 to 1.0)	0.14
MAKE28	148 (57.4)	139 (54.3)	3.1 (−5.5 to 11.6)	0.54
Need for new RRT	62 (24.1)	62 (24.1)	0.0 (−7.4 to 7.4)	> 0.99
Sustained renal failure at day 28 or ICU discharge if before 28 days	118 (45.7)	108 (42.0)	3.7 (−4.9 to 12.3)	0.45
Duration of RRT (days)	3.0 (1.0, 8.0)	5.0 (2.0, 9.0)	−2.0 (−3.0 to 0.0)	0.24
Length of ICU stays (days)				
All participants	7.3 (3.2, 14.8)	8.3 (3.9, 13.5)	−1.0 (−2.6 to 0.8)	0.66
Survivors	9.1 (5.0, 16.1)	9.0 (4.9, 14.1)	0.2 (−2.0 to 2.7)	0.31
Non-survivors	3.2 (1.4, 8.9)	5.7 (2.2, 11.7)	−2.6 (−5.7 to −0.8)	0.09
Length of hospital stay (days)				
All participants	19.6 (10.1, 40.4)	22.7 (11.7, 42.3)	−3.1 (−7.0 to 2.2)	0.24
Survivors	30.1 (16.8, 48)	27.7 (18, 52.3)	2.5 (−5.9 to 8.2)	0.81
Non-survivors	8.2 (3.4, 18.6)	11.3 (5.1, 25.7)	−3.1 (−6.5 to 0.7)	0.25
Safety outcomes				
Participants with a SAE (any)	32 (12.4)	23 (8.9)	3.5 (−2.3 to 9.2)	0.26
Subcategories of SAEs				
Any life-threatening arrhythmias	15 (5.8)	6 (2.3)	3.5 (−0.3 to 7.3)	0.08
Atrial fibrillation/supraventricular tachycardia	8 (3.1)	1 (0.4)	2.7 (0.1 to 5.3)	0.04

TABLE 13 Summary of clinical outcomes^a (continued)

Outcome	Treatment group, n(%)		Absolute difference (95% CI)	p-value ^a
	Levosimendan (N = 258)	Placebo (N = 257)		
Bradycardia	0 (0.0)	2 (0.8)	-0.8 (-2.2 to 0.7)	0.48
Ventricular fibrillation/tachycardia	7 (2.7)	3 (1.2)	1.5 (-1.2 to 4.3)	0.34
Myocardial infarction/acute coronary syndrome	3 (1.2)	1 (0.4)	0.8 (-1.1 to 2.7)	0.62
Other	18 (7.0)	17 (6.6)	-0.4 (-4.3 to 5.1)	> 0.99

Data shown as mean (SD) for continuous outcomes, median (lower quartile value, upper quartile value) for duration outcomes and n (%) for binary outcomes. CIs were calculated using bootstrap methods for all continuous variables.

a Continuous outcomes tested using a Mann–Whitney test; binary outcomes tested using a chi-squared test.

b Prespecified sensitivity analysis implementing Bayesian models using MCMC methods (see *Report Supplementary Material 1*).

c Represents 95% credible interval.

d One participant in the placebo group declined follow-up after ICU discharge but before day 28 and hospital discharge.

Tables 14 and 15 show the main model as specified in the supplementary SAP (see www.journalslibrary.nihr.ac.uk/programmes/eme/111408) for biomarker data, along with sensitivity analysis of a simpler model assuming that the treatment difference is constant over time (i.e. omitting the treatment*time interaction). For the model with no interaction, the estimated change over time applies to both the levosimendan arm and the placebo arm, and treatment differences are assumed to be constant over time. The fit of the models was compared using the deviance information criterion, values of which are provided in *Report Supplementary Material 1 (Tables S37–43)*.

As the assumption of linear change with time seemed reasonable for all measures, and given the lack of evidence that treatment effects varied over time, we did not consider the treatment differences at each time point separately. As the biomarkers were all log-transformed, the results are displayed as ratios in Tables 14 and 15 and expressed as percentage differences in the accompanying text. For all biomarkers, the results were not sensitive to adjustment for age and APACHE II score at baseline or to ICU effects. These results can be found in *Report Supplementary Material 1 (Tables S37–43)*.

N-terminal prohormone of brain natriuretic peptide

The results for NT-proBNP are shown in Table 14. NT-proBNP increased on average by 9% [95% credible interval (CrI) 0% to 19%] per day in the levosimendan group and decreased by 3% (95% CrI -10% to 5%) in the placebo group. The CrIs include 1, indicating that there is little evidence of longitudinal trends in either arm. The probability that NT-proBNP decreased faster in the levosimendan arm was 3% and the simpler model removing the treatment*time interaction fitted the data equally well (see *Report Supplementary Material 1, Tables S37*). There was some evidence of a treatment difference only on day 6 (26% difference, 95% CrI 2% to 54%). There were 113 NT-proBNP values below the lower limit of detection (32 of which occurred at baseline) and three above the upper limit of detection (two at baseline).

Troponin

The results for troponin are shown in Table 14. Troponin decreased over time in both arms: by 13% per day (95% CrI -26% to 1%) in the levosimendan arm and by 25% (95% CrI -35% to -14%) in the placebo arm. The probability that troponin decreased faster in the levosimendan arm was 8% and the model without the treatment*time interaction gave a similar fit. There was some evidence of a treatment difference only on day 6, but the CrI was wide (52% difference, 95% CrI 1% to 219%). There were no values outside the limits of detection for troponin.

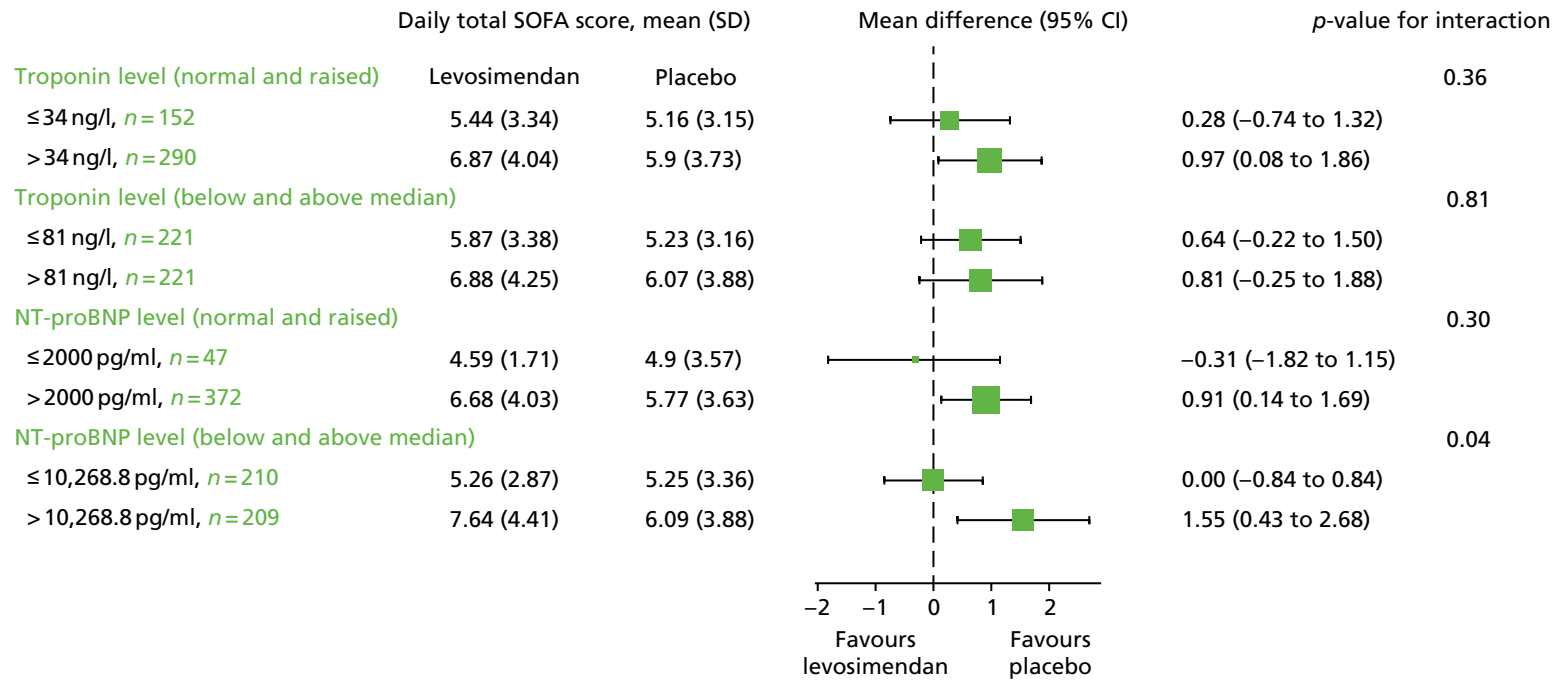


FIGURE 20 Forest plot showing the difference in mean total SOFA score, by subgroup based on cardiovascular biomarkers at baseline.

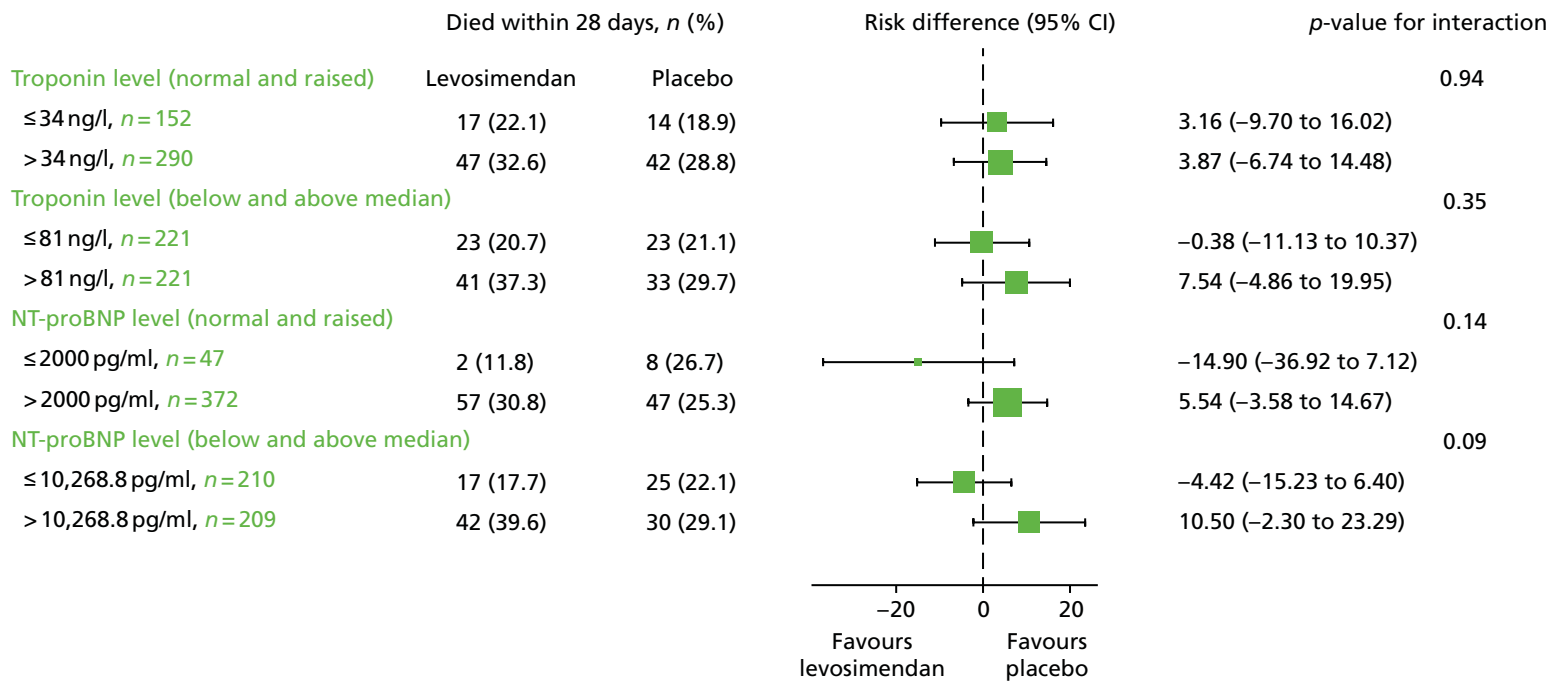


FIGURE 21 Forest plot showing the difference in 28-day mortality, by subgroup based on cardiovascular biomarkers at baseline.

TABLE 14 Estimated effects of levosimendan on cardiovascular biomarkers from longitudinal models

Variable	Biomarker			
	NT-proBNP		Troponin	
	Main model	No interaction	Main model	No interaction
Change per day – levosimendan	1.09 (1.00 to 1.19)	1.02 (0.96 to 1.09)	0.87 (0.74 to 1.01)	0.80 (0.72 to 0.89)
Change per day – placebo	0.97 (0.90 to 1.05)		0.75 (0.65 to 0.86)	
Pr(faster reduction in levosimendan)	0.032	–	0.082	–
Treatment difference on day 2	1.00 (0.84 to 1.19)	1.10 (0.94 to 1.27)	1.12 (0.79 to 1.55)	1.26 (0.92 to 1.67)
Treatment difference on day 4	1.12 (0.96 to 1.30)		1.30 (0.95 to 1.73)	
Treatment difference on day 6	1.26 (1.02 to 1.54)		1.52 (1.01 to 2.19)	

All differences are ratios of levosimendan compared with placebo. Data in parentheses are 95% CrIs.

Chemokine ligand 2

The results for CCL2 are shown in *Table 15*. CCL2 decreased over time in both arms: by 22% per day (95% CrI –27% to –17%) in the levosimendan arm and by 29% (95% CrI –34% to –25%) in the placebo arm. The probability that CCL2 decreased faster in the levosimendan arm was 2%, but again the simpler model with no interaction fitted the data equally well. There was little evidence of a treatment difference on any day. There were four values above the upper limit of detection, all of which were at baseline, and no values below the lower limit of detection.

Interleukin 6

The results for IL-6 are shown in *Table 15*. IL-6 decreased over time in both arms: by 50% (95% CrI –56% to –44%) per day in the levosimendan arm and also by 50% (95% CrI –55% to –44%) per day in the placebo arm. The probability that IL-6 decreased faster in the levosimendan arm was 54%. Removing the interaction term slightly improved the fit of the model and there was little evidence of a treatment difference on any day. There were 30 values above the upper limit of detection, 26 of which were at baseline.

Interleukin 8

The results for IL-8 are shown in *Table 15*. IL-8 decreased over time by 15% per day (95% CrI –20% to –9%) in the levosimendan arm and by 20% per day (95% CrI –25% to –16%) in the placebo arm. Removing the interaction term gave a similar model fit and there was little evidence of a treatment difference on any day. There were four values above the upper limit of detection, two of which were at baseline.

Interleukin 10

The results for IL-10 are shown in *Table 15*. IL-10 decreased over time by a similar amount in both arms (levosimendan: –32% per day, 95% CrI –37% to –27%; placebo: 31% per day, 95% CrI –35% to –26%). Removing the interaction term slightly improved the fit of the model. There was little evidence of a treatment difference on any day. There was one value above the upper limit of detection and one value below the lower limit of detection.

Soluble tumour necrosis factor receptor 1

The results for sTNFr1 are shown in *Table 15*. sTNFr1 decreased by the same amount per day in the placebo arm and the levosimendan arm (–10%, 95% CrI –14% to –7%) and the probability of a faster decrease in the placebo arm was therefore 50%. The simpler model with no treatment*time interaction had a slightly better fit. There was little evidence of a treatment effect on any day. There was one value above the upper limit of detection.

TABLE 15 Estimated effects of levosimendan on inflammatory biomarkers from longitudinal models

Variable	Biomarker									
	CCL2		IL-6		IL-8		IL-10		sTNFr1	
	Main model	No interaction	Main model	No interaction	Main model	No interaction	Main model	No interaction	Main model	No interaction
Change per day – levosimendan	0.78 (0.73, 0.83)	0.74 (0.71, 0.77)	0.50 (0.44, 0.56)	0.50 (0.46, 0.54)	0.85 (0.80, 0.91)	0.82 (0.79, 0.86)	0.68 (0.63, 0.73)	0.68 (0.65, 0.72)	0.90 (0.86, 0.93)	0.90 (0.87, 0.92)
Change per day – placebo	0.71 (0.66, 0.75)		0.50 (0.45, 0.56)		0.80 (0.75, 0.84)		0.69 (0.65, 0.74)		0.90 (0.86, 0.93)	
Pr(faster reduction in levosimendan)	0.019	–	0.536	–	0.046	–	0.662	–	0.500	–
Treatment difference on day 2	0.89 (0.78, 1.02)	0.96 (0.86, 1.07)	1.00 (0.79, 1.25)	0.99 (0.82, 1.20)	1.00 (0.85, 1.17)	1.06 (0.91, 1.22)	1.06 (0.90, 1.25)	1.05 (0.91, 1.20)	1.02 (0.93, 1.12)	1.02 (0.94, 1.11)
Treatment difference on day 4	0.98 (0.87, 1.10)		0.99 (0.81, 1.20)		1.08 (0.93, 1.24)		1.04 (0.90, 1.19)		1.02 (0.94, 1.11)	
Treatment difference on day 6	1.08 (0.92, 1.26)		0.99 (0.74, 1.29)		1.16 (0.97, 1.38)		1.02 (0.85, 1.23)		1.02 (0.92, 1.13)	

All differences are ratios of levosimendan compared with placebo.

Pharmacokinetic analysis

Forty-one participants in the levosimendan group were included in the pharmacokinetic analysis. The mean age of participants was 63.0 years and mean weight was 76.7 kg (Table 16).

In total, levosimendan and the two metabolites OR-1855 and OR-1896 were measured in 53, 148 and 103 plasma samples respectively (Table 17).

TABLE 16 Demographics of participants in the pharmacokinetic analysis

Characteristic	Mean	Median	SD
Age (years)	63.0	64.0	14.26
Weight (kg)	76.7	76.0	20.24
APACHE II score	23.3	23.5	7.29

TABLE 17 Numbers of pharmacokinetic samples at each time point

Time post dose (hours)	Levosimendan, <i>n</i>	Metabolites, <i>n</i>	
		OR-1855	OR-1896
Total number of samples			
22	44	44	44
66	34	34	34
144	28	28	28
192	28	28	28
240	21	21	21
312	14	14	14
384	17	17	17
Total	186	186	186
Measurable			
22	40	38	15
66	11	31	27
144	1	25	19
192	0	24	18
240	1	15	12
312	0	8	4
384	0	7	8
Total	53	148	103
Below limit of detection or not reportable			
22	4	6	29
66	23	3	7
144	27	3	9
192	28	4	10
240	20	6	9
312	14	6	10
384	17	10	9
Total	133	38	83

The lower limit of quantification in plasma is 0.1 ng/ml for levosimendan, 0.1 ng/ml for OR-1855 and 0.5 ng/ml for OR-1896.

Dosage adjustment occurred in line with the trial protocol. The mean patient dose of levosimendan was 18.2 mg during the 24-hour administration period (*Table 18*).

The AUC was calculated for individual patients using the trapezoid rule; it was calculated only for patients with at least three concentration measurements (*Table 19*). Data relating to assay results below the limit of quantification were excluded from the analyses (*Figures 22 and 23*).

The concentration–time profiles for levosimendan and both metabolites (OR-1855 and OR-1896) are shown in *Figures 24–26*, respectively. Levosimendan undergoes rapid clearance in the first 48 hours. The peak concentration of both metabolites occurs at 6 days, with both metabolites detectable at 16 days. The mean maximum OR-1855 concentration was 2.8 mg/l, with an AUC of 615.9 mg × hour/l (see *Figure 25*). The mean maximum OR-1896 concentration was 4.0 mg/l, with an AUC of 962.9 mg × hour/l (see *Figure 26*).

There was a linear relationship between the maximum OR-1855 and OR-1896 concentrations for each patient (Pearson's $r = 0.60$; $p = 0.0004$) and the OR-1855 and OR-1896 AUC for each patient (Pearson's $r = 0.58$; $p = 0.0035$) (*Figure 27*).

The impact of renal function (peak creatinine level) and RRT is shown in *Figures S12–S16* (see *Report Supplementary Material 1*). No significant differences in metabolite exposure and the use of RRT were found. The influence of liver function (liver component of the SOFA score) on peak metabolite concentration and the AUC is shown in *Figures S17 and S18* (see *Report Supplementary Material 1*); no effects on metabolite levels were found.

TABLE 18 Levosimendan dose

Levosimendan dose	Mean	Median	SD
mg/24 hours	18.200	18.600	8.10
µg/kg/minute	0.167	0.198	0.06

TABLE 19 Pharmacokinetic parameters of levosimendan and the two metabolites (OR-1855 and OR-1896)

Parameter	Mean	Median	IQR
Maximum levosimendan concentration (mg/l)	30.9	28.4	21.0
Maximum OR-1855 concentration (mg/l)	2.6	1.1	1.9
OR-1855 AUC (mg × hour/l)	397.4	126.6	327.4
Maximum OR-1896 concentration (mg/l)	4.0	1.6	5.1
OR-1896 AUC (mg × hour/l)	679.1	394.6	1005.9

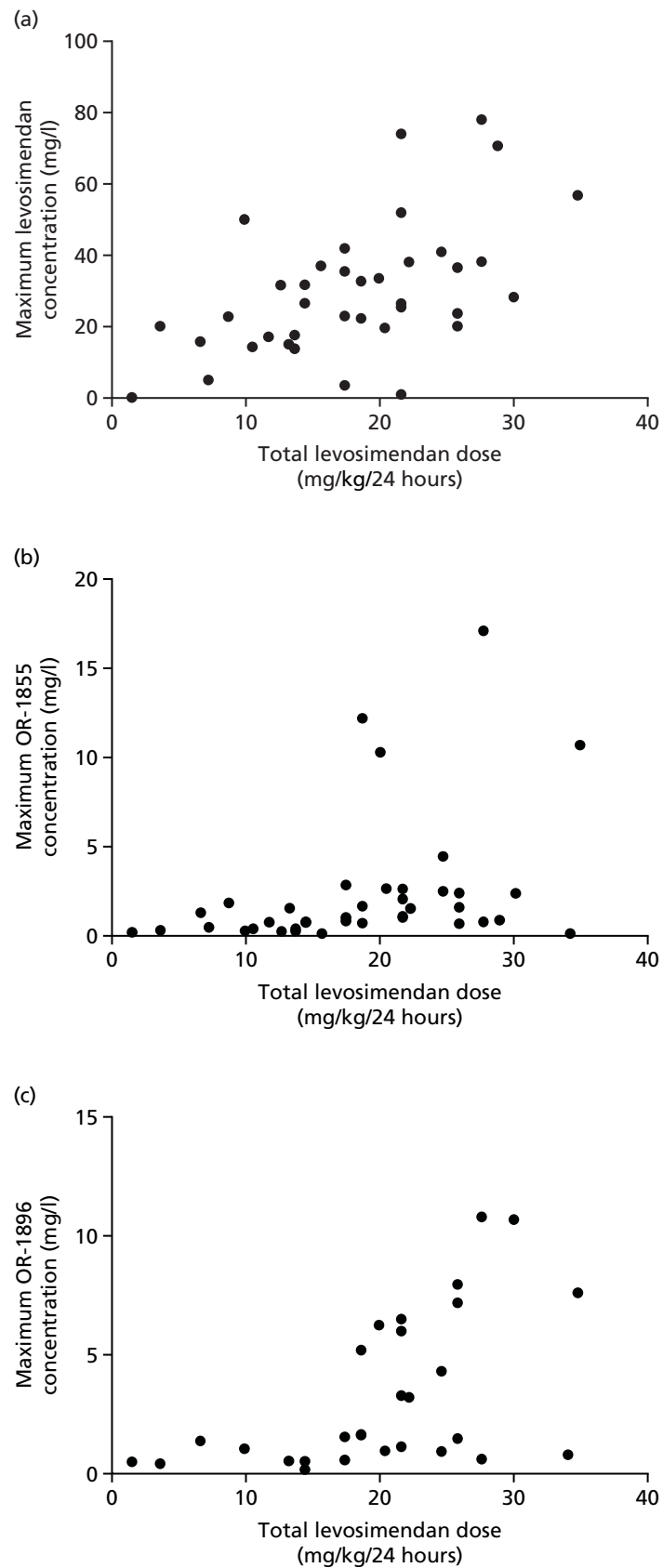


FIGURE 22 Relationship between total levosimendan dose (mg/kg/24 hours) and maximum levosimendan concentration (mg/l) (a), maximum OR-1855 concentration (mg/l) (b) and maximum OR-1896 concentration (mg/l) (c).

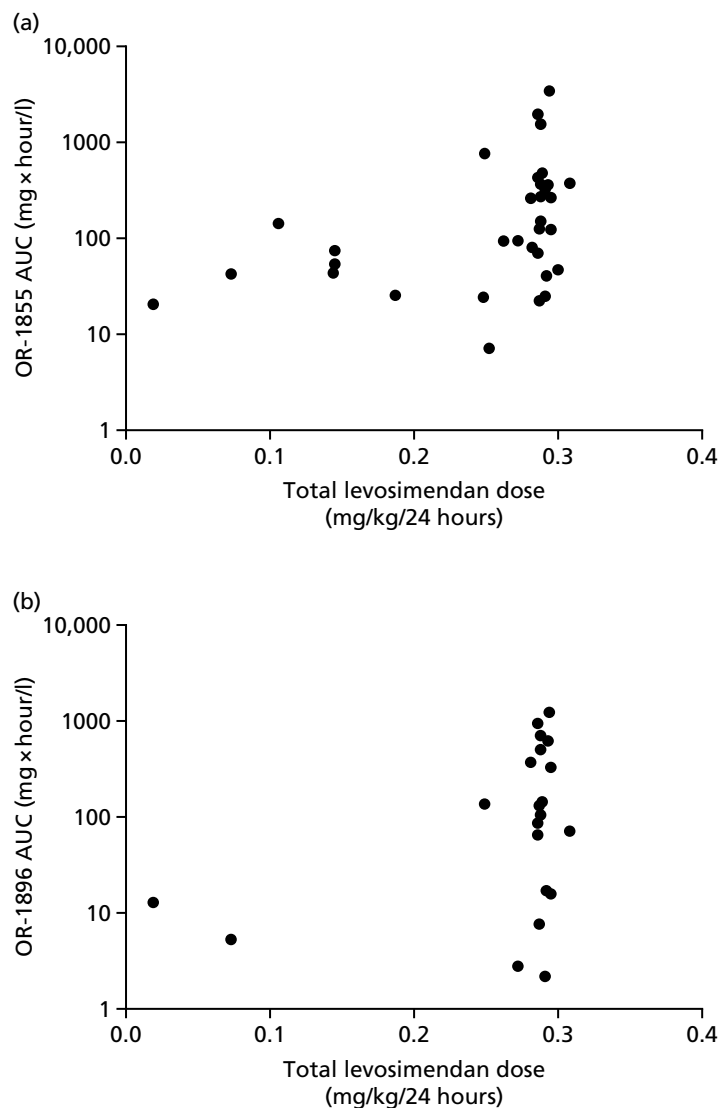


FIGURE 23 Relationship between total levosimendan dose (mg/kg/24 hours) and OR-1855 AUC (mg × hour/l) (a) and OR-1896 AUC (mg × hour/l) (b).

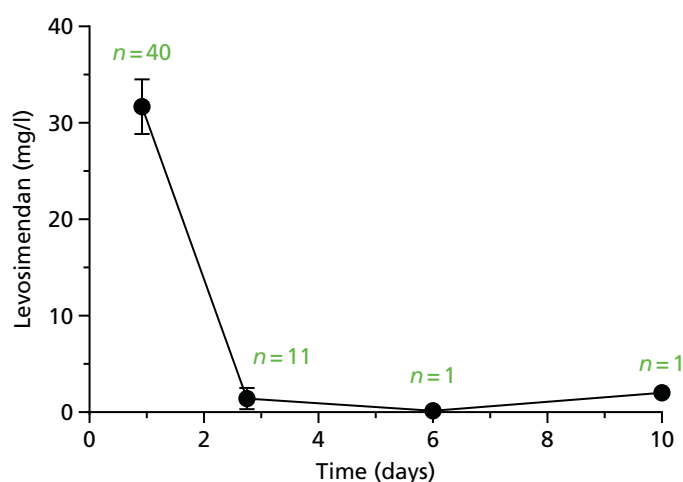


FIGURE 24 Mean levosimendan concentration following infusion. The range is the standard error of the mean; n is the number of observations at each time point.

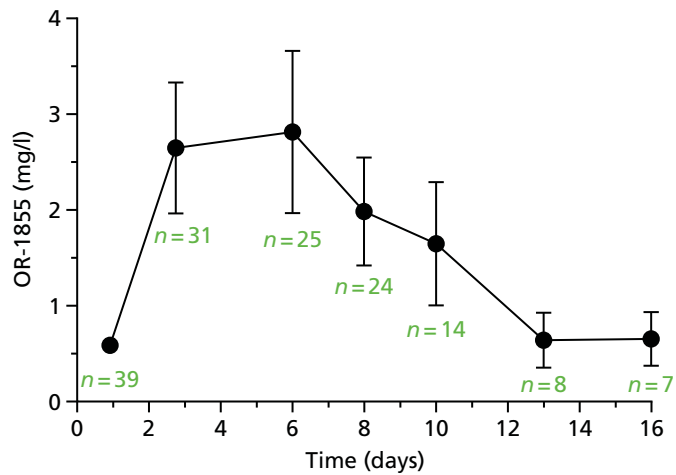


FIGURE 25 Mean OR-1855 concentration following levosimendan infusion. The range is the standard error of the mean; *n* is the number of observations at each time point.

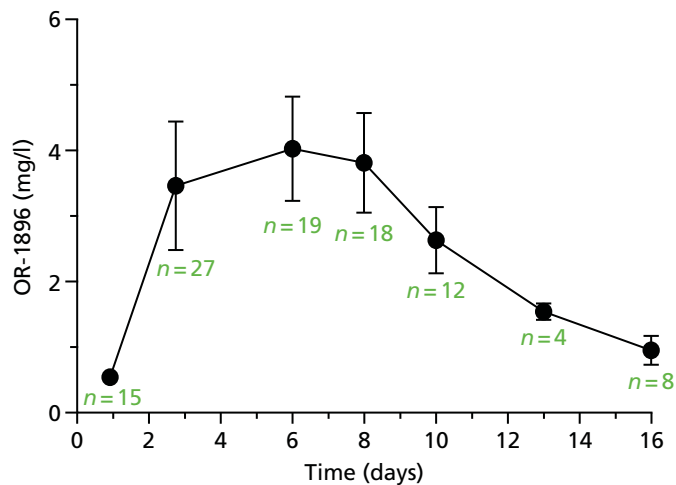


FIGURE 26 Mean OR-1896 concentration following levosimendan infusion. The range is the standard error of the mean; *n* is the number of observations at each time point.

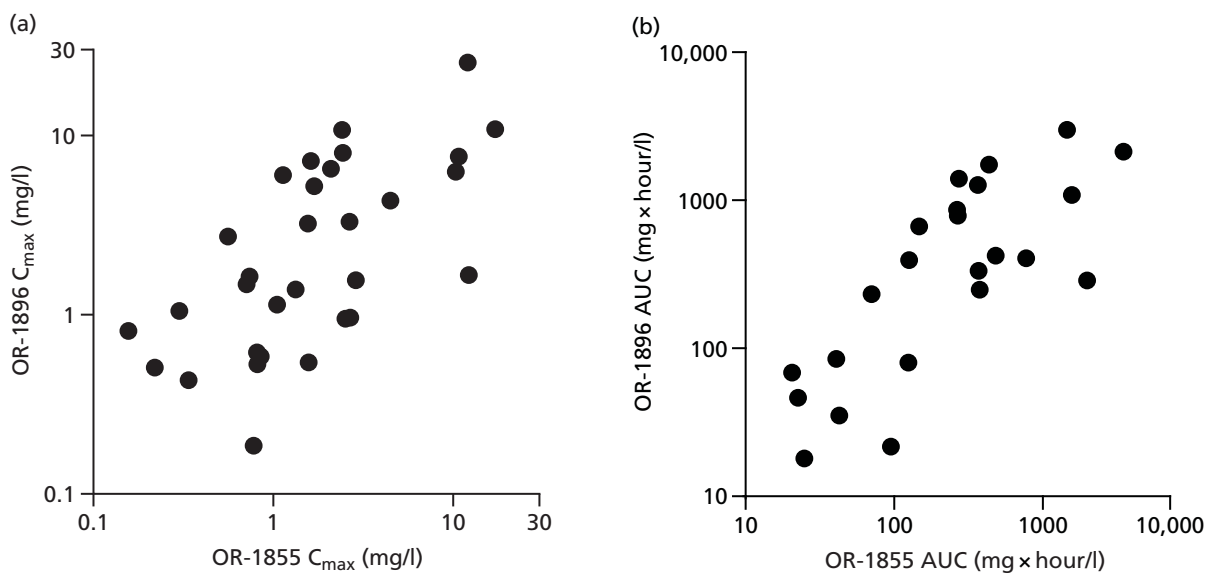


FIGURE 27 Relationship between the maximum OR-1855 and OR-1896 concentrations for each patient (a) and the maximum OR-1855 AUC and OR-1896 AUC for each patient (b).

Chapter 5 Discussion

In this multicentre, double-blind randomised clinical trial levosimendan did not reduce organ dysfunction or mortality when added to standard care for adult patients who had septic shock. Patients treated with levosimendan required more noradrenaline, had a higher heart rate and required mechanical ventilation for longer. There was also a higher rate of supraventricular tachycardias.

Cardiovascular resuscitation is an essential element of sepsis management.⁴⁶ However, there is ever-increasing evidence that high doses of catecholamine infusions^{16,66} and high circulating levels of catecholamines are associated with worse outcomes and more AEs.¹⁷ Alternative non-catecholamine vasopressor and inotrope options are thus being investigated.⁶⁶ Levosimendan produces its inotropic action through different mechanisms from the mechanism of action of catecholamines. By sensitising cardiomyocytes to existing levels of intracellular calcium, an increased myocardial contraction is achieved with a minimal increase in myocardial oxygen demand. This is in contrast to catecholamines, which increase the myocardial oxygen demand.⁶⁷ As calcium levels fall in diastole, relaxation of the myocardium is not impaired with levosimendan, and this lusitropic effect may be an additional benefit over catecholamines.⁶⁸ Although levosimendan has a half-life of about 1 hour, its active metabolite OR-1896 has a long half-life, and a single 24-hour infusion of levosimendan should provide haemodynamic effects over a week,⁶⁹ long enough to cover the majority of cases of septic shock.⁷⁰

Levosimendan may have other important non-inotropic effects. It opens ATP-sensitive potassium channels in vascular smooth muscle leading to vasodilatation. It may also be protective to the heart and other organs, especially in ischaemia/reperfusion injury.^{9,71} Additional potential properties of levosimendan, including anti-inflammatory,⁵² antioxidative¹⁰ and anti-apoptotic effects,⁷² have all been described.

In view of these pleiotropic effects beyond just inotropy, combined with the fact that myocardial dysfunction is present in > 50% of the septic shock population,¹² it may not be evident at the onset of shock and it may also go unrecognised even using cardiac output monitoring,⁷³ we decided to recruit all patients who had septic shock. However, four subgroup analyses were planned to examine the effect of levosimendan in higher risk patients, including those with a low cardiac output, those with impaired oxygen delivery to the tissues and those on high doses of catecholamines. There was no evidence of a beneficial effect of levosimendan in any of these prespecified subgroups with regard to either mean SOFA scores or mortality.

A number of preclinical and small clinical trials have demonstrated a potential benefit of levosimendan for renal,³⁰ liver³⁵ and pulmonary³¹ function in sepsis. Therefore, the mean daily SOFA score was chosen in this study as the primary outcome to assess the effect of levosimendan on multiple organ function. However, there was no evidence of any beneficial effect on the total SOFA score, either in the whole study population or in any of the predefined subgroups. No benefit was seen for any individual component of the SOFA score nor for any other clinical outcome. The cardiovascular SOFA score was higher in the levosimendan group, reflecting the higher doses of noradrenaline required to maintain MAP.

The detailed analyses of physiological variables over time also did not demonstrate any clear evidence of clinical benefit with regard to individual organ function. Not surprisingly, the cardiac index and central venous saturations tended to be higher in the levosimendan group, reflecting its known inotropic effect. However, this did not lead to any benefits for other organ function, such as liver or kidney function. Although levosimendan does not stimulate beta-adrenoreceptors, we saw a significantly higher heart rate in the levosimendan group, most likely because of vasodilatation but possibly related to the increased requirement for noradrenaline.⁶³ Although noradrenaline is an alpha-adrenoreceptor agonist causing vasoconstriction, it does have some beta-adrenoreceptor agonist effects and may increase the heart rate compared with non-catecholamine vasoconstrictors.⁶³ Similarly, there was a higher rate of tachyarrhythmias in levosimendan-treated patients. These may have contributed to the lack of overall clinical benefit, which is consistent with data suggesting a potential benefit of treating persistent tachycardia in sepsis using beta-blockers.⁷⁴ This hypothesis is currently being investigated in the STRESS-L [STudy into the REversal of

Septic Shock with Landiolol (Beta Blockade)] trial (NIHR EME reference no. 14/150/85, EudraCT number 2017-001785-14; ISRCTN12600919).

Interestingly, the levosimendan group was less likely to be successfully weaned from mechanical ventilation over 28 days. Levosimendan has been reported to sensitise diaphragmatic muscle to calcium, improve contractility and reverse the development of fatigue after muscle loading.⁴⁸ Combined with the prolonged inotropic effect of levosimendan and its active metabolite, levosimendan might have been expected to improve weaning from mechanical ventilation. Why the opposite effect was observed remains unclear, especially as there was no difference in intravenous fluid administration nor fluid balance between treatment groups. It is possible that the increased use of noradrenaline in the levosimendan group may have contributed to further catecholamine-induced myocardial dysfunction. The PaO_2/FiO_2 ratio was also lower in the levosimendan participants on day 1 but there was then a more rapid improvement over time after this. It is possible that any vasodilatation in the first 24 hours may have contributed to an early ventilation/perfusion mismatch.

We also carried out analyses on inflammatory biomarkers over time to assess levosimendan's anti-inflammatory effects, which have previously been described.⁵² Despite measuring a number of both pro- and anti-inflammatory markers, we found no clear effect of levosimendan on these circulating biomarkers.

Limitations

There were limitations to this study that must be considered. This was a trial of levosimendan added to standard care rather than a comparison of levosimendan against an alternative inotrope such as dobutamine. Fewer than 10% of patients in the standard care group received dobutamine. However, there was no difference in outcome (mean SOFA score or mortality) in the prespecified subgroup analysis of patients with a low cardiac index. It should be noted that the number of patients with a measured low cardiac index was small ($n = 52$) and therefore the study lacks power to fully assess the effect of levosimendan in this subgroup of patients. Similarly, no echocardiographic analyses were performed to provide more detailed information about changes in myocardial function with levosimendan treatment. Therefore, this trial cannot provide guidance as to which inotrope is best for use in the management of sepsis if a low cardiac index is present and an inotrope is being considered. However, we did measure troponin as a marker of myocardial injury and NT-proBNP as a marker of ventricular dysfunction. Surprisingly, those patients with the highest troponin and NT-proBNP levels tended to have higher mean SOFA scores and mortality when treated with levosimendan. Similar to the tachycardia effect described earlier, it is not clear if this is caused by levosimendan directly or is the consequence of the increased noradrenaline use, but it does provide further support for the concept that low cardiac output states in sepsis should not be routinely treated with inotropic drugs.

The dose of levosimendan in this trial was titrated between 0.05 and 0.2 $\mu\text{g}/\text{kg}/\text{minute}$ in an attempt to administer the maximum tolerated dose. However, it has been reported that a dose of 0.1 $\mu\text{g}/\text{kg}/\text{minute}$ may be as effective as 0.2 $\mu\text{g}/\text{kg}/\text{minute}$ and may avoid excessive hypotension.⁷⁵ The pharmacokinetic analysis revealed that plasma levels of the parent drug and its active metabolites were very similar to levels seen in other patient cohorts and, importantly, there was no effect of acute kidney or liver failure on these drug levels. This suggests that the dosing regimen used in this trial was appropriate.

It is also worth noting that the target MAP of 65–70 mmHg recommended in the protocol and reiterated regularly at all investigator meetings was frequently exceeded. This is commonly seen in other shock trials^{61,68} and suggests that the noradrenaline doses administered could have been reduced in both treatment groups.

Future research work

Although this study found no evidence of any clinically meaningful benefits of levosimendan for patients with septic shock, it is still unclear if there is any role for inotropic therapy in the management of septic shock in patients with an extremely low cardiac output. Future studies could compare levosimendan against dobutamine and against placebo to test if there is any benefit from either inotrope for this specific but limited indication.

Although the use of the mean SOFA score as the primary outcome in this study has provided very clear answers about the effects of levosimendan, it is not a perfect outcome measure for a Phase II trial. It is not a patient-centred outcome. However, there are limitations to all currently used outcomes in Phase II trials in sepsis. Future work should investigate the different possible outcomes and highlight their relative strengths and weaknesses.

Conclusions

Among adult patients with septic shock levosimendan when added to standard care does not reduce organ dysfunction or mortality. Patients allocated to the levosimendan group required more noradrenaline, were less likely to be successfully weaned from mechanical ventilation and had more tachycardia and a higher rate of supraventricular arrhythmias, but there were no overall differences in SAEs between the groups.

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Publications

Orme RM, Perkins GD, McAuley DF, Liu KD, Mason AJ, Morelli A, *et al.* An efficacy and mechanism evaluation study of Levosimendan for the Prevention of Acute Organ Dysfunction in Sepsis (LeoPARDS): protocol for a randomized controlled trial. *Trials* 2014;**15**:199.

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Data-sharing statement

All data requests should be submitted to the corresponding author for consideration. Access to anonymised data may be granted following review.

Patient data

This work uses data provided by patients and collected by the NHS as part of their care and support. Using patient data is vital to improve health and care for everyone. There is huge potential to make better use of information from people's patient records, to understand more about disease, develop new treatments, monitor safety, and plan NHS services. Patient data should be kept safe and secure, to protect everyone's privacy, and it's important that there are safeguards to make sure that it is stored and used responsibly. Everyone should be able to find out about how patient data are used. #datasaveslives You can find out more about the background to this citation here: <https://understandingpatientdata.org.uk/data-citation>.

References

1. Harrison DA, Welch CA, Eddleston JM. The epidemiology of severe sepsis in England, Wales and Northern Ireland, 1996 to 2004: secondary analysis of a high quality clinical database, the ICNARC Case Mix Programme Database. *Crit Care* 2006;**10**:R42. <https://doi.org/10.1186/cc4854>
2. 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. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. *Intensive Care Med* 1996;**22**:707–10. <https://doi.org/10.1007/BF01709751>
3. Ferreira FL, Bota DP, Bross A, Mélot C, Vincent JL. Serial evaluation of the SOFA score to predict outcome in critically ill patients. *JAMA* 2001;**286**:1754–8. <https://doi.org/10.1001/jama.286.14.1754>
4. Oppert M, Engel C, Brunkhorst FM, Bogatsch H, Reinhart K, Frei U, *et al.* Acute renal failure in patients with severe sepsis and septic shock – a significant independent risk factor for mortality: results from the German Prevalence Study. *Nephrol Dial Transplant* 2008;**23**:904–9. <https://doi.org/10.1093/ndt/gfm610>
5. Davies A, Green C, Hutton J, Chinn C. Severe sepsis: a European estimate of the burden of disease in ICU. *Intensive Care Med* 2001;**27**(Suppl. 2):S284.
6. Gheorghiade M, Teerlink JR, Mebazaa A. Pharmacology of new agents for acute heart failure syndromes. *Am J Cardiol* 2005;**96**:68G–73G. <https://doi.org/10.1016/j.amjcard.2005.07.023>
7. Sorsa T, Pollesello P, Solaro RJ. The contractile apparatus as a target for drugs against heart failure: interaction of levosimendan, a calcium sensitiser, with cardiac troponin c. *Mol Cell Biochem* 2004;**266**:87–107. <https://doi.org/10.1023/B:MCBI.0000049141.37823.19>
8. Papp Z, Edes I, Fruhwald S, De Hert SG, Salmenpera M, Leppikangas H, *et al.* Levosimendan: molecular mechanisms and clinical implications: consensus of experts on the mechanisms of action of levosimendan. *Int J Cardiol* 2012;**159**:82–7. <https://doi.org/10.1016/j.ijcard.2011.07.022>
9. Buckley JF, Singer M, Clapp LH. Role of KATP channels in sepsis. *Cardiovasc Res* 2006;**72**:220–30. <https://doi.org/10.1016/j.cardiores.2006.07.011>
10. Hasslacher J, Bijuklic K, Bertocchi C, Kountchev J, Bellmann R, Dunzendorfer S, Joannidis M. Levosimendan inhibits release of reactive oxygen species in polymorphonuclear leukocytes in vitro and in patients with acute heart failure and septic shock: a prospective observational study. *Crit Care* 2011;**15**:R166. <https://doi.org/10.1186/cc10307>
11. Kankaanranta H, Zhang X, Tumelius R, Ruotsalainen M, Haikala H, Nissinen E, Moilanen E. Antieosinophilic activity of simendans. *J Pharmacol Exp Ther* 2007;**323**:31–8. <https://doi.org/10.1124/jpet.107.124057>
12. Vieillard-Baron A, Caille V, Charron C, Belliard G, Page B, Jardin F. Actual incidence of global left ventricular hypokinesia in adult septic shock. *Crit Care Med* 2008;**36**:1701–6. <https://doi.org/10.1097/CCM.0b013e318174db05>
13. Rudiger A, Singer M. Mechanisms of sepsis-induced cardiac dysfunction. *Crit Care Med* 2007;**35**:1599–608. <https://doi.org/10.1097/01.CCM.0000266683.64081.02>
14. Dünser MW, Hasibeder WR. Sympathetic overstimulation during critical illness: adverse effects of adrenergic stress. *J Intensive Care Med* 2009;**24**:293–316. <https://doi.org/10.1177/0885066609340519>
15. Annane D, Vignon P, Renault A, Bollaert PE, Charpentier C, Martin C, *et al.* Norepinephrine plus dobutamine versus epinephrine alone for management of septic shock: a randomised trial. *Lancet* 2007;**370**:676–84. [https://doi.org/10.1016/S0140-6736\(07\)61344-0](https://doi.org/10.1016/S0140-6736(07)61344-0)

16. Dünser MW, Ruokonen E, Pettilä V, Ulmer H, Torgersen C, Schmittinger CA, *et al.* Association of arterial blood pressure and vasopressor load with septic shock mortality: a post hoc analysis of a multicenter trial. *Crit Care* 2009;**13**:R181. <https://doi.org/10.1186/cc8167>
17. Boldt J, Menges T, Kuhn D, Diridis C, Hempelmann G. Alterations in circulating vasoactive substances in the critically ill – a comparison between survivors and non-survivors. *Intensive Care Med* 1995;**21**:218–25. <https://doi.org/10.1007/BF01701475>
18. Rehberg S, Ertmer C, Vincent JL, Spiegel HU, Köhler G, Erren M, *et al.* Effects of combined arginine vasopressin and levosimendan on organ function in ovine septic shock. *Crit Care Med* 2010;**38**:2016–23. <https://doi.org/10.1097/CCM.0b013e3181ef4694>
19. Zager RA, Johnson AC, Lund S, Hanson SY, Abrass CK. Levosimendan protects against experimental endotoxemic acute renal failure. *Am J Physiol Renal Physiol* 2006;**290**:F1453–62. <https://doi.org/10.1152/ajprenal.00485.2005>
20. Fries M, Ince C, Rossaint R, Bleilevens C, Bickenbach J, Rex S, *et al.* Levosimendan but not norepinephrine improves microvascular oxygenation during experimental septic shock. *Crit Care Med* 2008;**36**:1886–91. <https://doi.org/10.1097/CCM.0b013e31817cede9>
21. Dubin A, Murias G, Sottile JP, Pozo MO, Barán M, Edul VS, *et al.* Effects of levosimendan and dobutamine in experimental acute endotoxemia: a preliminary controlled study. *Intensive Care Med* 2007;**33**:485–94. <https://doi.org/10.1007/s00134-006-0519-5>
22. García-Septien J, Lorente JA, Delgado MA, de Paula M, Nin N, Moscoso A, *et al.* Levosimendan increases portal blood flow and attenuates intestinal intramucosal acidosis in experimental septic shock. *Shock* 2010;**34**:275–80. <https://doi.org/10.1097/SHK.0b013e3181cd8c5b>
23. Cunha-Goncalves D, Perez-de-Sa V, Grins E, Dahm PL, Thörne J, Blomquist S. Inotropic support during experimental endotoxemic shock: part I. The effects of levosimendan on splanchnic perfusion. *Anesth Analg* 2009;**109**:1568–75. <https://doi.org/10.1213/ane.0b013e3181af3fe3>
24. Cunha-Goncalves D, Perez-de-Sa V, Larsson A, Thörne J, Blomquist S. Inotropic support during experimental endotoxemic shock: part II. A comparison of levosimendan with dobutamine. *Anesth Analg* 2009;**109**:1576–83. <https://doi.org/10.1213/ane.0b013e3181af40e0>
25. De Backer D, Bracht H. Levosimendan in early sepsis: when good ideas give poor results. *Anesth Analg* 2009;**109**:1367–9. <https://doi.org/10.1213/ANE.0b013e3181b9e91a>
26. Noto A, Giacomini M, Palandi A, Stabile L, Reali-Forster C, Iapichino G. Levosimendan in septic cardiac failure. *Intensive Care Med* 2005;**31**:164–5. <https://doi.org/10.1007/s00134-004-2502-3>
27. Matejovic M, Krouzecky A, Radej J, Novak I. Successful reversal of resistant hypodynamic septic shock with levosimendan. *Acta Anaesthesiol Scand* 2005;**49**:127–8. <https://doi.org/10.1111/j.1399-6576.2005.00541.x>
28. Ramaswamykanive H, Bihari D, Solano TR. Myocardial depression associated with pneumococcal septic shock reversed by levosimendan. *Anaesth Intensive Care* 2007;**35**:409–13.
29. Powell BP, De Keulenaer BL. Levosimendan in septic shock: a case series. *Br J Anaesth* 2007;**99**:447–8. <https://doi.org/10.1093/bja/aem225>
30. Morelli A, De Castro S, Teboul JL, Singer M, Rocco M, Conti G, *et al.* Effects of levosimendan on systemic and regional hemodynamics in septic myocardial depression. *Intensive Care Med* 2005;**31**:638–44. <https://doi.org/10.1007/s00134-005-2619-z>
31. Morelli A, Teboul JL, Maggiore SM, Vieillard-Baron A, Rocco M, Conti G, *et al.* Effects of levosimendan on right ventricular afterload in patients with acute respiratory distress syndrome: a pilot study. *Crit Care Med* 2006;**34**:2287–93. <https://doi.org/10.1097/01.CCM.0000230244.17174.4F>

32. Morelli A, Donati A, Ertmer C, Rehberg S, Lange M, Orecchioni A, *et al.* Levosimendan for resuscitating the microcirculation in patients with septic shock: a randomized controlled study. *Crit Care* 2010;**14**:R232. <https://doi.org/10.1186/cc9387>
33. Alhashemi JA, Alotaibi QA, Abdullah GM, Shalabi SA. Levosimendan vs dobutamine in septic shock. *J Crit Care* 2009;**24**:e14–5. <https://doi.org/10.1016/j.jcrc.2009.06.006>
34. Vaitsis J, Michalopoulou H, Thomopoulos C. Use of levosimendan in myocardial dysfunction due to sepsis. *Crit Care* 2009;**13**(S1):P165. <https://doi.org/10.1186/cc7329>
35. Memis D, Inal MT, Sut N. The effects of levosimendan vs dobutamine added to dopamine on liver functions assessed with noninvasive liver function monitoring in patients with septic shock. *J Crit Care* 2012;**27**:318.e1-6. <https://doi.org/10.1016/j.jcrc.2011.06.008>
36. Morelli A, Ertmer C, Rehberg S. Effects of levosimendan on renal function in septic shock: a case control study. *Crit Care* 2009;**13**(S1):P170. <https://doi.org/10.1186/cc7334>
37. Yilmaz MB, Yalta K, Yontar C, Karadas F, Erdem A, Turgut OO, *et al.* Levosimendan improves renal function in patients with acute decompensated heart failure: comparison with dobutamine. *Cardiovasc Drugs Ther* 2007;**21**:431–5. <https://doi.org/10.1007/s10557-007-6066-7>
38. Pinto BB, Rehberg S, Ertmer C, Westphal M. Role of levosimendan in sepsis and septic shock. *Curr Opin Anaesthesiol* 2008;**21**:168–77. <https://doi.org/10.1097/ACO.0b013e3282f43c56>
39. Reinhart K, Brunkhorst FM, Bone HG, Bardutzky J, Dempfle CE, Forst H, *et al.* Prevention, diagnosis, therapy and follow-up care of sepsis: 1st revision of S-2k guidelines of the German Sepsis Society (Deutsche Sepsis-Gesellschaft e.V. (DSG)) and the German Interdisciplinary Association of Intensive Care and Emergency Medicine (Deutsche Interdisziplinäre Vereinigung für Intensiv- und Notfallmedizin (DIVI)). *Ger Med Sci* 2010;**8**:Doc14. <https://doi.org/10.3205/000103>
40. Alberti C, Brun-Buisson C, Goodman SV, Guidici D, Granton J, Moreno R, *et al.* Influence of systemic inflammatory response syndrome and sepsis on outcome of critically ill infected patients. *Am J Respir Crit Care Med* 2003;**168**:77–84. <https://doi.org/10.1164/rccm.200208-785OC>
41. Orme RM, Perkins GD, McAuley DF, Liu KD, Mason AJ, Morelli A, *et al.* An efficacy and mechanism evaluation study of Levosimendan for the Prevention of Acute oRgan Dysfunction in Sepsis (LeoPARDS): protocol for a randomized controlled trial. *Trials* 2014;**15**:199. <https://doi.org/10.1186/1745-6215-15-199>
42. Gordon AC, Perkins GD, Singer M, McAuley DF, Orme RM, Santhakumaran S, *et al.* Levosimendan for the prevention of acute organ dysfunction in sepsis. *N Engl J Med* 2016;**375**:1638–48. <https://doi.org/10.1056/NEJMoa1609409>
43. Brunkhorst FM, Engel C, Bloos F, Meier-Hellmann A, Ragaller M, Weiler N, *et al.* Intensive insulin therapy and pentastarch resuscitation in severe sepsis. *N Engl J Med* 2008;**358**:125–39. <https://doi.org/10.1056/NEJMoa070716>
44. 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**:124–34. <https://doi.org/10.1056/NEJMoa1204242>
45. Myburgh JA, Finfer S, Bellomo R, Billot L, Cass A, Gattas D, *et al.* Hydroxyethyl starch or saline for fluid resuscitation in intensive care. *N Engl J Med* 2012;**367**:1901–11. <https://doi.org/10.1056/NEJMoa1209759>
46. 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. *Crit Care Med* 2013;**41**:580–637. <https://doi.org/10.1097/CCM.0b013e31827e83af>

47. Mehta RL, Kellum JA, Shah SV, Molitoris BA, Ronco C, Warnock DG, Levin A, Acute Kidney Injury Network. Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. *Crit Care* 2007;**11**:R31. <https://doi.org/10.1186/cc5713>
48. Doorduyn J, Sinderby CA, Beck J, Stegeman DF, van Hees HW, van der Hoeven JG, Heunks LM. The calcium sensitizer levosimendan improves human diaphragm function. *Am J Respir Crit Care Med* 2012;**185**:90–5. <https://doi.org/10.1164/rccm.201107-1268OC>
49. Palevsky PM, Molitoris BA, Okusa MD, Levin A, Waikar SS, Wald R, et al. Design of clinical trials in acute kidney injury: report from an NIDDK workshop on trial methodology. *Clin J Am Soc Nephrol* 2012;**7**:844–50. <https://doi.org/10.2215/CJN.12791211>
50. Charpentier J, Luyt CE, Fulla Y, Vinsonneau C, Cariou A, Grabar S, et al. Brain natriuretic peptide: a marker of myocardial dysfunction and prognosis during severe sepsis. *Crit Care Med* 2004;**32**:660–5. <https://doi.org/10.1097/01.CCM.0000114827.93410.D8>
51. Post F, Weilemann LS, Messow CM, Sinning C, Münzel T. B-type natriuretic peptide as a marker for sepsis-induced myocardial depression in intensive care patients. *Crit Care Med* 2008;**36**:3030–7. <https://doi.org/10.1097/CCM.0b013e31818b9153>
52. Wang Q, Yokoo H, Takashina M, Sakata K, Ohashi W, Abedelzaher LA, et al. Anti-inflammatory profile of levosimendan in cecal ligation-induced septic mice and in lipopolysaccharide-stimulated macrophages. *Crit Care Med* 2015;**43**:e508–20. <https://doi.org/10.1097/CCM.0000000000001269>
53. Famous KR, Delucchi K, Ware LB, Kangelaris KN, Liu KD, Thompson BT, Calfee CS, ARDS Network. Acute respiratory distress syndrome subphenotypes respond differently to randomized fluid management strategy. *Am J Respir Crit Care Med* 2017;**195**:331–8. <https://doi.org/10.1164/rccm.201603-0645OC>
54. Gordon AC, Mason AJ, Thirunavukkarasu N, Perkins GD, Cecconi M, Cepkova M, et al. Early vasopressin vs norepinephrine on kidney failure in patients with septic shock: the VANISH randomized clinical trial. *JAMA* 2016;**316**:509–18. <https://doi.org/10.1001/jama.2016.10485>
55. Daniels MJ, Hogan JW. *Missing Data in Longitudinal Studies: Strategies for Bayesian Modeling and Sensitivity Analysis*. New York, NY: Chapman & Hall/CRC; 2008.
56. Anderson MJ, Ter Braak CJF. Permutation tests for multi-factorial analysis of variance. *J Stat Comput Sim* 2003;**73**:85–113. <https://doi.org/10.1080/00949650215733>
57. Henderson R, Diggle P, Dobson A. Joint modelling of longitudinal measurements and event time data. *Biostatistics* 2000;**1**:465–80. <https://doi.org/10.1093/biostatistics/1.4.465>
58. Gordon AC, Mason AJ, Perkins GD, Stotz M, Terblanche M, Ashby D, Brett SJ. The interaction of vasopressin and corticosteroids in septic shock: a pilot randomized controlled trial. *Crit Care Med* 2014;**42**:1325–33. <https://doi.org/10.1097/CCM.0000000000000212>
59. Al-Mohammad A, Mant J, Laramie P, Swain S, Chronic Heart Failure Guideline Development Group. Diagnosis and management of adults with chronic heart failure: summary of updated NICE guidance. *BMJ* 2010;**341**:c4130. <https://doi.org/10.1136/bmj.c4130>
60. Harvey S, Harrison DA, Singer M, Ashcroft J, Jones CM, Elbourne D, et al. Assessment of the clinical effectiveness of pulmonary artery catheters in management of patients in intensive care (PAC-Man): a randomised controlled trial. *Lancet* 2005;**366**:472–7. [https://doi.org/10.1016/S0140-6736\(05\)67061-4](https://doi.org/10.1016/S0140-6736(05)67061-4)
61. Russell JA, Walley KR, Singer J, Gordon AC, Hébert PC, Cooper DJ, et al. Vasopressin versus norepinephrine infusion in patients with septic shock. *N Engl J Med* 2008;**358**:877–87. <https://doi.org/10.1056/NEJMoa067373>

62. Gordon AC, Russell JA, Walley KR, Singer J, Ayers D, Storms MM, *et al.* The effects of vasopressin on acute kidney injury in septic shock. *Intensive Care Med* 2010;**36**:83–91. <https://doi.org/10.1007/s00134-009-1687-x>
63. Gordon AC, Wang N, Walley KR, Ashby D, Russell JA. The cardio-pulmonary effects of vasopressin compared with norepinephrine in septic shock. *Chest* 2012;**142**:593–605. <https://doi.org/10.1378/chest.11-2604>
64. Moreno R, Sprung CL, Annane D, Chevret S, Briegel J, Keh D, *et al.* Time course of organ failure in patients with septic shock treated with hydrocortisone: results of the Corticus study. *Intensive Care Med* 2011;**37**:1765–72. <https://doi.org/10.1007/s00134-011-2334-x>
65. World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. *JAMA* 2013;**310**:2191–4. <https://doi.org/10.1001/jama.2013.281053>
66. Singer M. Catecholamine treatment for shock – equally good or bad? *Lancet* 2007;**370**:636–7. [https://doi.org/10.1016/S0140-6736\(07\)61317-8](https://doi.org/10.1016/S0140-6736(07)61317-8)
67. Ukkonen H, Saraste M, Akkila J, Knuuti MJ, Lehtikainen P, Nägren K, *et al.* Myocardial efficiency during calcium sensitization with levosimendan: a noninvasive study with positron emission tomography and echocardiography in healthy volunteers. *Clin Pharmacol Ther* 1997;**61**:596–607. [https://doi.org/10.1016/S0009-9236\(97\)90139-9](https://doi.org/10.1016/S0009-9236(97)90139-9)
68. Zangrillo A, Putzu A, Monaco F, Oriani A, Frau G, De Luca M, *et al.* Levosimendan reduces mortality in patients with severe sepsis and septic shock: a meta-analysis of randomized trials. *J Crit Care* 2015;**30**:908–13. <https://doi.org/10.1016/j.jcrc.2015.05.017>
69. Kivikko M, Antila S, Eha J, Lehtonen L, Pentikäinen PJ. Pharmacokinetics of levosimendan and its metabolites during and after a 24-hour continuous infusion in patients with severe heart failure. *Int J Clin Pharmacol Ther* 2002;**40**:465–71. <https://doi.org/10.5414/CP40465>
70. Jörgensen K, Bech-Hanssen O, Houltz E, Ricksten SE. Effects of levosimendan on left ventricular relaxation and early filling at maintained preload and afterload conditions after aortic valve replacement for aortic stenosis. *Circulation* 2008;**117**:1075–81. <https://doi.org/10.1161/CIRCULATIONAHA.107.722868>
71. du Toit EF, Genis A, Opie LH, Pollesello P, Lochner A. A role for the RISK pathway and K(ATP) channels in pre- and post-conditioning induced by levosimendan in the isolated guinea pig heart. *Br J Pharmacol* 2008;**154**:41–50. <https://doi.org/10.1038/bjp.2008.52>
72. Parissis JT, Adamopoulos S, Antoniadis C, Kostakis G, Rigas A, Kyrzopoulos S, *et al.* Effects of levosimendan on circulating pro-inflammatory cytokines and soluble apoptosis mediators in patients with decompensated advanced heart failure. *Am J Cardiol* 2004;**93**:1309–12. <https://doi.org/10.1016/j.amjcard.2004.01.073>
73. Shahul S, Gulati G, Hacker MR, Mahmood F, Canelli R, Nizamuddin J, *et al.* Detection of myocardial dysfunction in septic shock: a speckle-tracking echocardiography study. *Anesth Analg* 2015;**121**:1547–54. <https://doi.org/10.1213/ANE.0000000000000943>
74. Morelli A, Ertmer C, Westphal M, Rehberg S, Kampmeier T, Ligges S, *et al.* Effect of heart rate control with esmolol on hemodynamic and clinical outcomes in patients with septic shock: a randomized clinical trial. *JAMA* 2013;**310**:1683–91. <https://doi.org/10.1001/jama.2013.278477>
75. de Geus HR, Bakker J, Lesaffre EM, le Noble JL. Neutrophil gelatinase-associated lipocalin at ICU admission predicts for acute kidney injury in adult patients. *Am J Respir Crit Care Med* 2011;**183**:907–14. <https://doi.org/10.1164/rccm.200908-1214OC>

Appendix 1 Additional information

Screening data

TABLE 20 Summary of screening data for all trial sites

Site	Screened patients (n)	Treatment group, number of randomised patients		Protocol deviations (n)
		Levosimendan	Placebo	
Altnagelvin Hospital	55	5	5	1
Antrim Area Hospital	51	17	17	3
Birmingham Heartlands	48	19	17	10
Bradford Royal Infirmary	19	6	8	6
Charing Cross Hospital	131	13	13	5
Cheltenham General	26	3	3	2
City General Hospital	17	2	4	1
Derriford Hospital	56	4	2	0
Gloucestershire Royal	3	0	1	0
Hammersmith Hospital	100	7	7	2
Hull Royal Infirmary	91	8	7	5
Ipswich Hospital	59	3	3	3
James Cook University Hospital	234	3	3	3
James Paget University Hospital	28	0	2	0
Kettering General Hospital	35	9	9	5
Leicester Royal Infirmary	43	8	8	0
Manchester Royal Infirmary	33	11	10	4
Medway Maritime Hospital	54	4	5	2
Musgrove Park Hospital	27	5	5	1
Norfolk and Norwich University Hospital	39	8	6	5
Pinderfields Hospital	18	2	2	0
Poole Hospital	118	13	13	4
Queen's Medical Centre	147	9	11	6
Queen Alexandra Hospital	160	7	7	1
Queen Elizabeth Hospital	60	9	9	1
Royal Cornwall Hospital	55	5	7	2
Royal Free Hospital	24	0	1	0

continued

TABLE 20 Summary of screening data for all trial sites (continued)

Site	Screened patients (n)	Treatment group, number of randomised patients		Protocol deviations (n)
		Levosimendan	Placebo	
Royal Preston Hospital	36	6	3	0
Royal Victoria Hospital	59	17	16	3
St James's Hospital	38	13	12	7
York Hospital	46	7	6	1
University College Hospital	268	24	24	9
University Hospital of South Manchester	17	4	3	2
Whiston Hospital	187	7	8	6
Total	2382	258	257	100

Accrual data

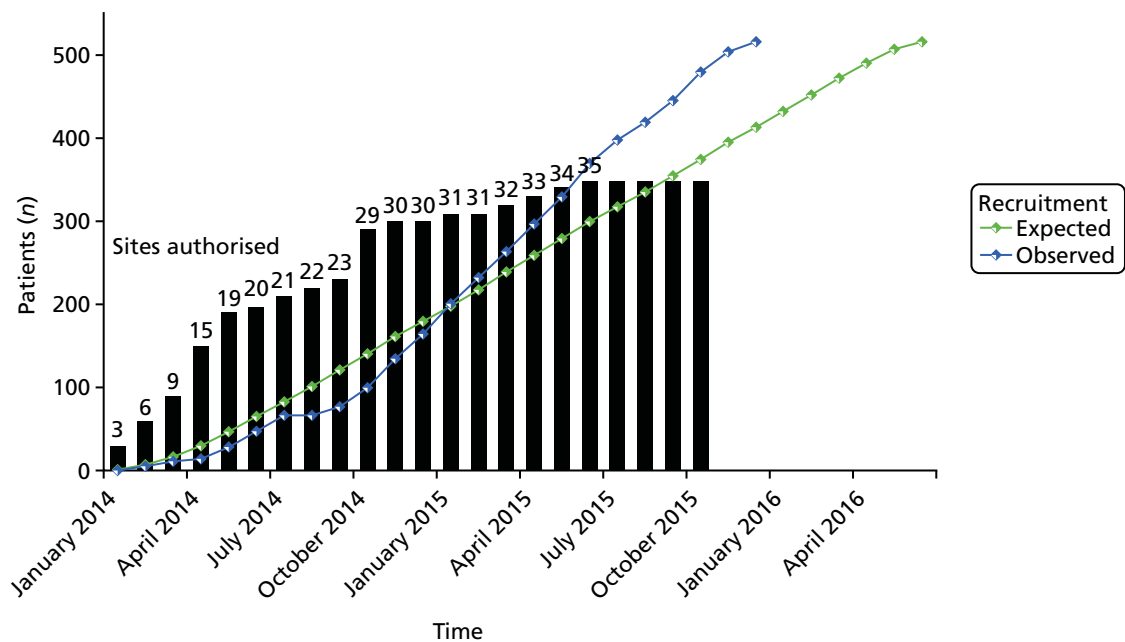


FIGURE 28 Recruitment of patients into the LeOPARDS trial.

Missing baseline data

TABLE 21 Number of patients with missing baseline characteristics

Characteristic	Treatment group (n)		Total
	Levosimendan	Placebo	
BMI	6	3	9
Organ failure			
Respiratory	1	1	2
Haematological	2	2	4
Liver	6	5	11
Neurological	34	45	79
Renal	0	1	1
Source or infection	1	0	1
Heart rhythm	0	2	2
Physiological variables			
Central venous pressure	66	77	143
SaO ₂	1	5	6
ScvO ₂	85	87	172
Lactate	3	2	5
PaO ₂ /FIO ₂	1	1	2
Creatinine	0	2	2
Bilirubin	6	5	11
Platelets	2	2	4
GCS score	34	45	79

BMI, body mass index; GCS, Glasgow Coma Scale.

TABLE 22 Completeness of SOFA score data by component and treatment group

SOFA component	Treatment group (n)							
	Levosimendan				Placebo			
	Subjects		Scores		Subjects		Scores	
	n/N	%	n/N	%	n/N	%	n/N	%
Respiration	53/258	20.5	104/2662	3.9	46/257	17.9	115/2693	4.3
Coagulation	116/258	45.0	182/2662	6.8	114/257	44.4	223/2693	8.3
Liver	151/258	58.5	340/2662	12.8	132/257	51.4	336/2693	13.6
Cardiovascular	51/258	19.8	60/2662	2.3	47/257	18.3	72/2693	2.7
Renal	92/258	35.7	141/2662	5.3	83/257	32.3	170/2693	6.3
Total SOFA score	175/258	67.8	441/2662	16.6	166/257	64.6	511/2693	19.0

TABLE 23 Mean total SOFA scores between randomisation and ICU discharge: as-treated analysis

SOFA component	Treatment group						Absolute difference (levosimendan – placebo)	
	Levosimendan			Placebo			Mean (95% CI) ^a	Median (95% CI) ^a
	Mean	SD	Median (LQ, UQ)	Mean	SD	Median (LQ, UQ)		
Respiration	1.69	1.18	1.57 (0.75, 2.67)	1.55	1.14	1.5 (0.50, 2.29)	0.14 (–0.07 to 0.34)	0.07 (–0.22 to 0.51)
Coagulation	0.75	1.06	0.14 (0.00, 1.18)	0.74	1.02	0.30 (0.00, 1.08)	0.01 (–0.17 to 0.19)	–0.16 (–0.33 to 0.12)
Liver	0.50	0.84	0.00 (0.00, 0.74)	0.44	0.76	0.00 (0.00, 0.58)	0.06 (–0.08 to 0.20)	0.00 (0.00 to 0.00)
Cardiovascular	2.26	1.19	2.00 (1.23, 3.44)	2.00	1.19	1.67 (1.00, 3.00)	0.26 (0.06 to 0.47)	0.33 (0.07 to 0.62)
Renal	1.46	1.49	1.00 (0.00, 2.86)	1.26	1.37	0.70 (0.00, 2.25)	0.19 (–0.05 to 0.44)	0.30 (–0.28 to 0.68)
Total	6.66	3.96	5.62 (3.75, 9.00)	5.99	3.84	4.89 (3.33, 7.67)	0.67 (–0.01 to 1.35)	0.72 (–0.25 to 1.25)

LQ, lower quartile values; UQ, upper quartile values.

a Calculated using bootstrapping.

TABLE 24 Mean total SOFA scores excluding the cardiovascular component between randomisation and ICU discharge

SOFA score	Treatment group						Absolute difference (levosimendan – placebo)	
	Levosimendan			Placebo			Mean (95% CI) ^a	Median (95% CI) ^a
	Mean	SD	Median (LQ, UQ)	Mean	SD	Median (LQ, UQ)		
Total	4.41	3.13	3.52 (2.15, 5.99)	4.05	3.07	3.36 (2, 5.29)	0.36 (–0.17 to 0.90)	0.17 (–0.36 to 0.78)

LQ, lower quartile values; UQ, upper quartile values.

a Calculated using bootstrapping.

TABLE 25 Mean total SOFA scores between randomisation and ICU discharge: ITT, Bayesian analysis

SOFA component	Treatment group				Absolute difference (levosimendan – placebo)
	Levosimendan		Placebo		
	Mean	SD	Mean	SD	Mean (95% CrI) ^a
Respiration	1.88	1.23	1.78	1.18	0.10 (–0.11 to 0.31)
Coagulation	0.83	1.08	0.86	1.09	–0.03 (–0.22 to 0.16)
Liver	0.49	0.82	0.48	0.81	0.01 (–0.13 to 0.15)
Cardiovascular	2.41	1.14	2.21	1.16	0.20 (0.00 to 0.39)
Renal	1.58	1.54	1.44	1.44	0.14 (–0.12 to 0.40)
Total	7.19	3.72	6.78	3.74	0.41 (–0.24 to 1.06)

a CrIs calculated using bootstrapping.

TABLE 26 Mean total SOFA scores between randomisation and ICU discharge by treatment: post hoc sensitivity analysis

Analysis	Treatment group						Absolute difference (levosimendan – placebo)	
	Levosimendan			Placebo			Mean (95% CI) ^a	Median (95% CI) ^a
	Mean	SD	Median (LQ, UQ)	Mean	SD	Median (LQ, UQ)		
Sensitivity 1 ^b	5.79	4.69	4.25 (2.07, 9.00)	5.21	4.48	3.86 (1.71, 7.68)	0.58 (-0.21 to 1.37)	0.39 (-0.57 to 1.41)
Sensitivity 2 ^b	5.34	5.08	3.16 (1.07, 9.05)	4.65	4.92	2.36 (0.89, 7.75)	0.69 (-0.18 to 1.56)	0.80 (-0.32 to 2.25)

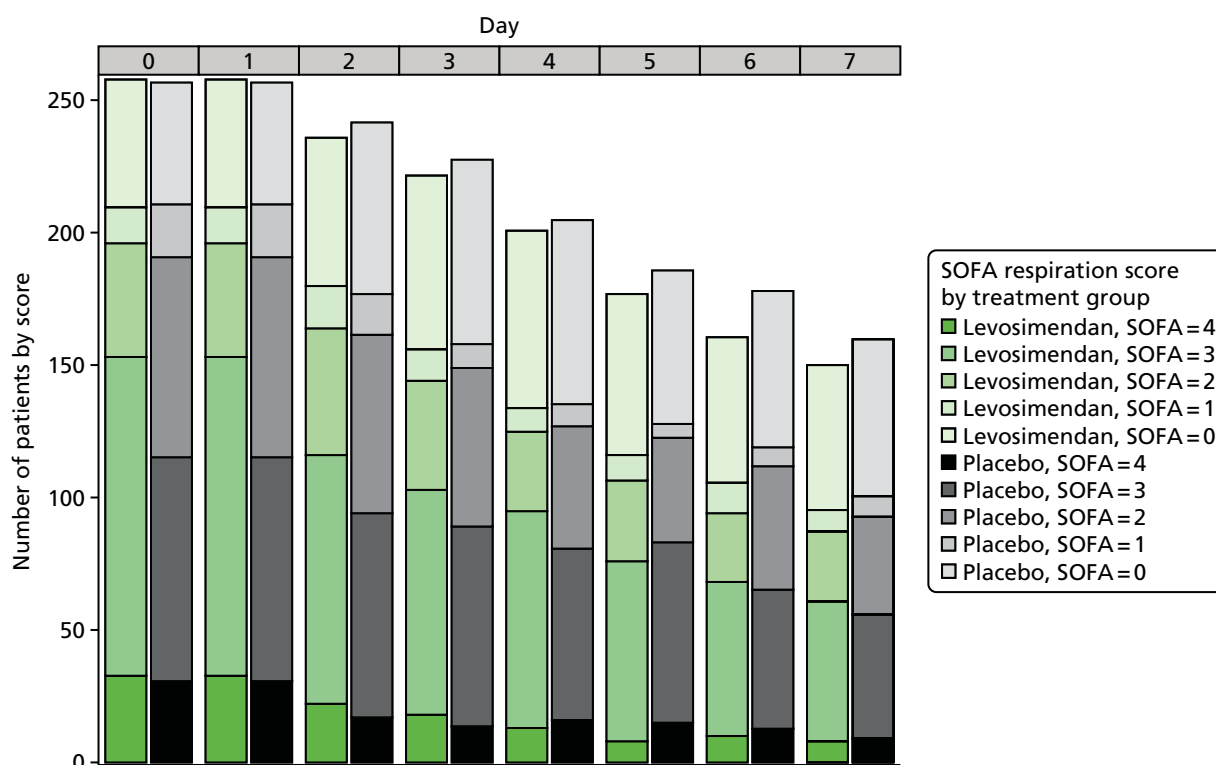
LQ, lower quartile values; UQ, upper quartile values.

^a Calculated using bootstrapping.

^b Sensitivity 1: the last recorded score was carried forward to all days after ICU discharge or death, up to day 28. Sensitivity 2: all days alive outside the ICU were assigned the minimum possible score of zero and all days dead were assigned the maximum score obtained by the patient in question.

Primary outcome data

Exploratory analysis

**FIGURE 29** Bar plot of respiratory SOFA scores, by day and treatment group.

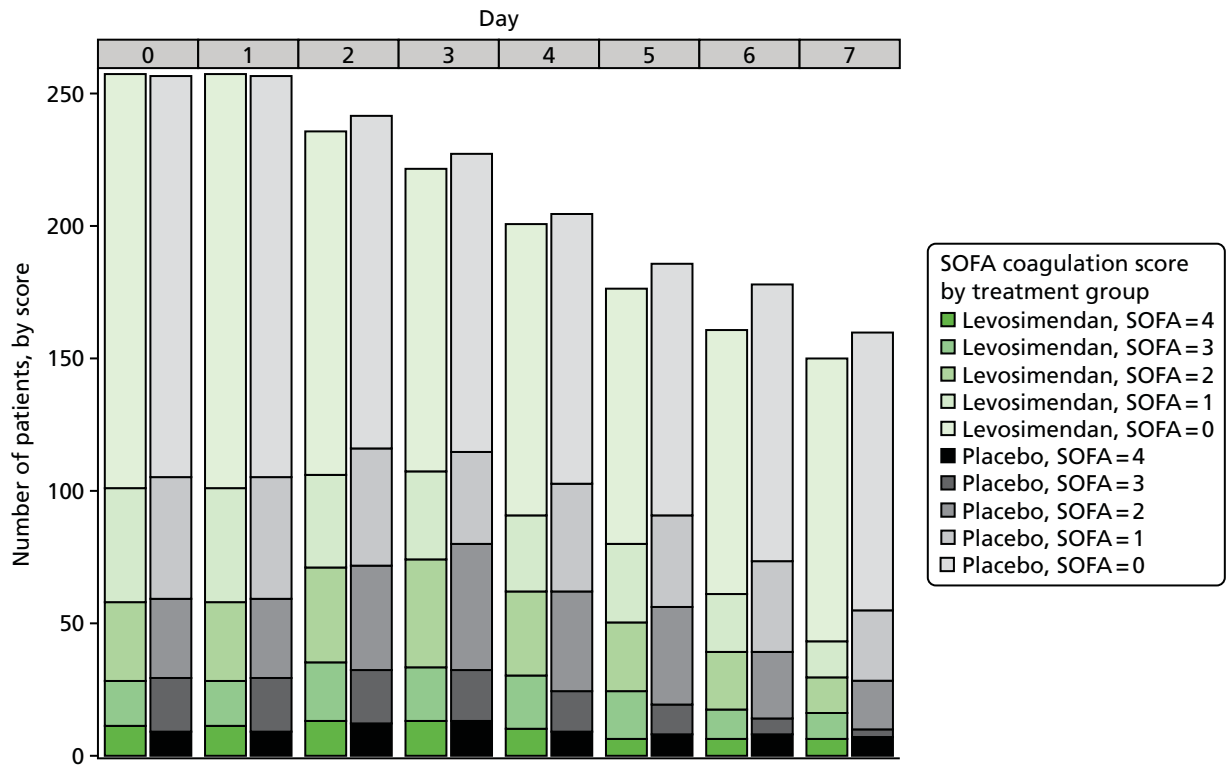


FIGURE 30 Bar plot of coagulation SOFA scores, by day and treatment group.

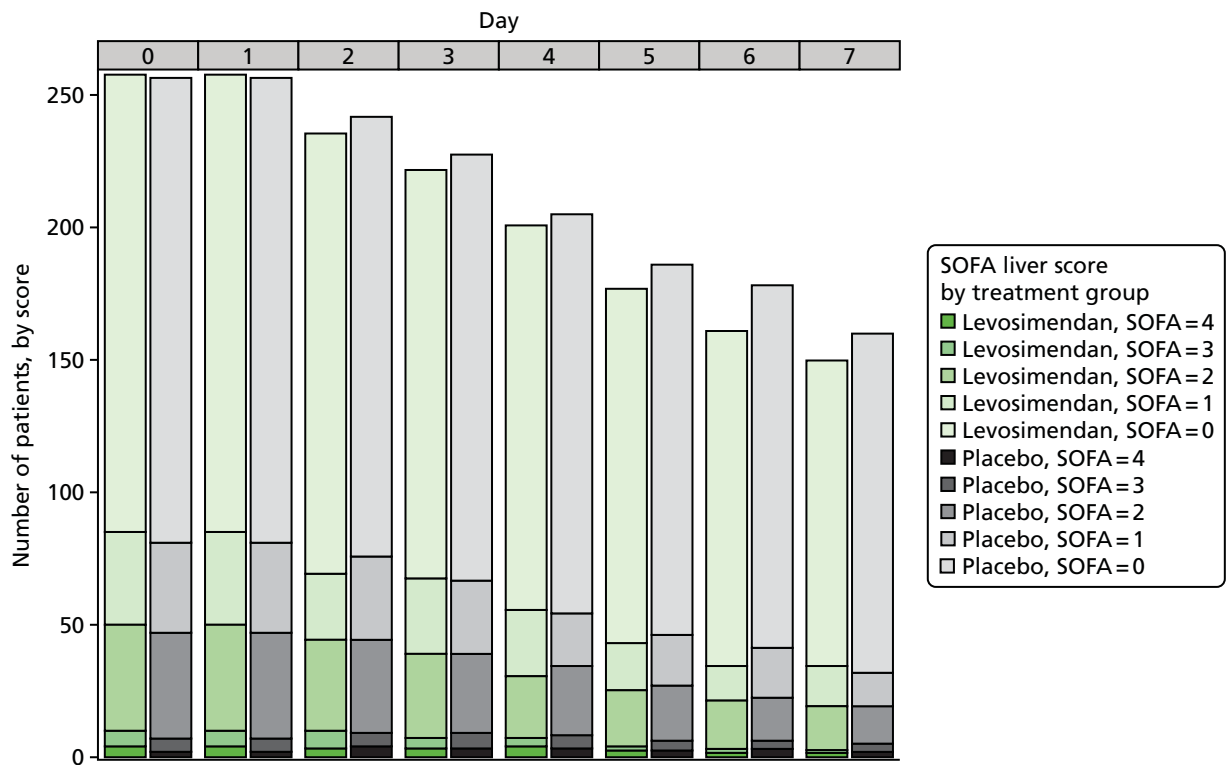


FIGURE 31 Bar plot of liver SOFA scores, by day and treatment group.

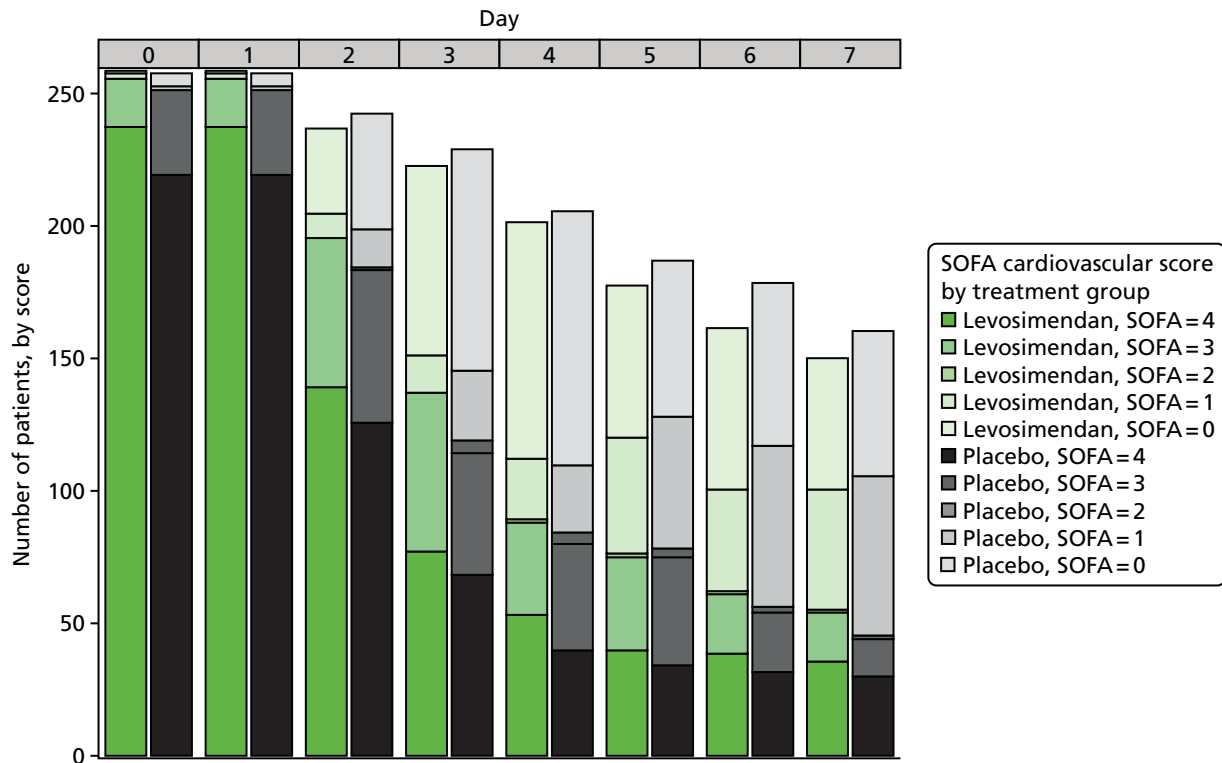


FIGURE 32 Bar plot of cardiovascular SOFA scores, by day and treatment group.

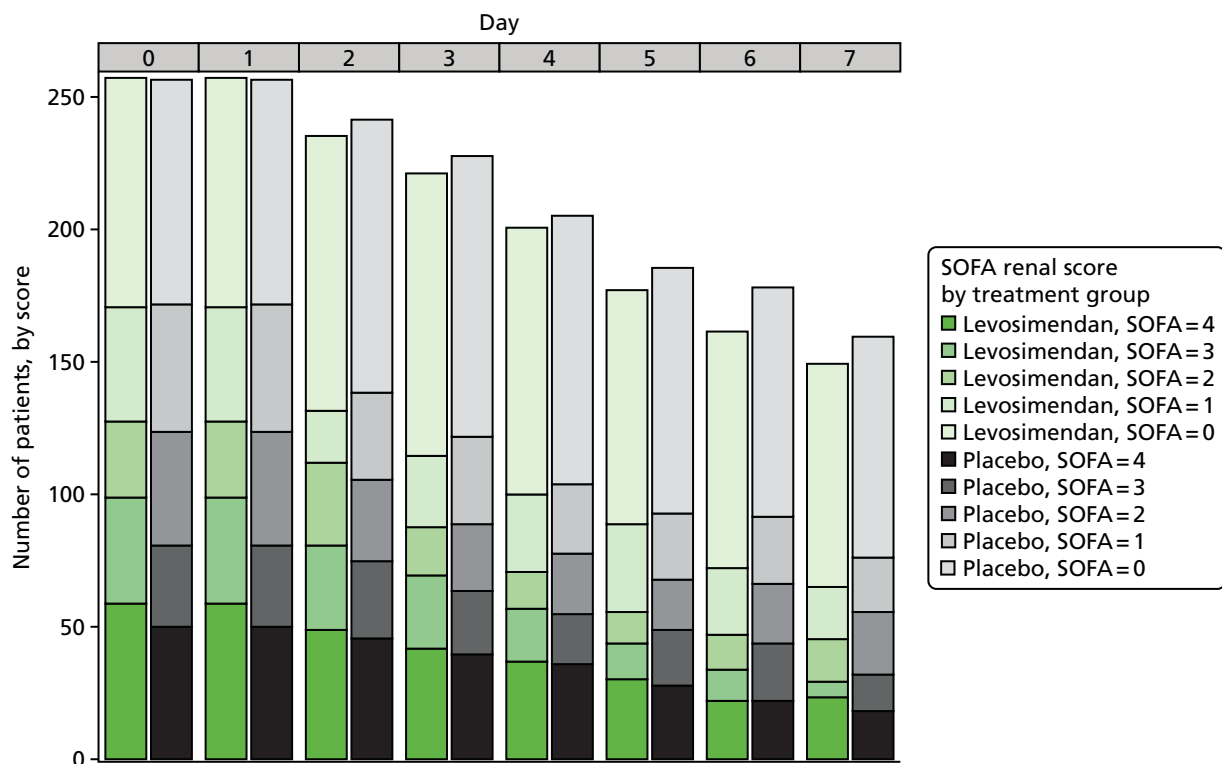


FIGURE 33 Bar plot of renal SOFA scores, by day and treatment group.

Subgroup analysis

Cardiac index

Patients were split into the lowest tertile for baseline cardiac index (≤ 2.44 l/minute/m², 52 patients) and the middle and highest tertiles combined (> 2.44 l/minute/m², 102 patients).

Tables 27 and 28 show the mean total SOFA scores for the lowest tertile and the middle and highest tertiles combined, respectively.

TABLE 27 Mean total SOFA score between randomisation and ICU discharge: lowest tertile of baseline cardiac index

SOFA component	Treatment group						Absolute difference (levosimendan – placebo)	
	Levosimendan			Placebo			Mean (95% CI) ^a	Median (95% CI) ^b
	Mean	SD	Median (LQ, UQ)	Mean	SD	Median (LQ, UQ)		
Respiration	2.16	1.05	2.12 (1.21, 3)	2.02	1.01	2.47 (1.07, 2.97)	0.13 (–0.42 to 0.69)	–0.36 (–0.89 to 1.00)
Coagulation	0.98	1.28	0.38 (0.00, 1.65)	1.23	1.30	1.00 (0.18, 1.71)	–0.25 (–0.96 to 0.44)	–0.62 (–1.09 to 0.45)
Liver	0.65	0.91	0.00 (0.00, 1.21)	0.71	0.94	0.00 (0.00, 1.75)	–0.06 (–0.56 to 0.44)	0.00 (–1.05 to 0.75)
Cardiovascular	2.87	1.31	3.81 (1.5, 4.00)	2.49	1.31	2.88 (1.27, 3.89)	0.39 (–0.32 to 1.09)	0.94 (–1.18 to 2.58)
Renal	2.00	1.64	1.88 (0.35, 3.74)	2.25	1.24	2.16 (1.47, 3.27)	–0.26 (–1.02 to 0.51)	–0.28 (–1.75 to 1.40)
Total	8.66	4.86	7.88 (4.12, 12.27)	8.71	4.11	7.25 (5.83, 10.88)	–0.04 (–2.45 to 2.33)	0.62 (–4.27 to 4.21)

LQ, lower quartile; UQ, upper quartile.

a Calculated using bootstrapping.

b Presented on the square root scale as there is no suitable back-transform to an interpretable scale.

TABLE 28 Mean total SOFA score between randomisation and ICU discharge: middle and highest tertiles of baseline cardiac index combined

SOFA component	Treatment group						Absolute difference (levosimendan – placebo)	
	Levosimendan			Placebo			Mean (95% CI) ^a	Median (95% CI) ^b
	Mean	SD	Median (LQ, UQ)	Mean	SD	Median (LQ, UQ)		
Respiration	1.95	1.20	2.05 (1.00, 3.00)	1.76	1.14	1.60 (1, 2.39)	0.19 (–0.25 to 0.63)	0.45 (–0.35 to 1.29)
Coagulation	0.79	0.95	0.50 (0.00, 1.22)	0.66	0.91	0.17 (0, 1.04)	0.13 (–0.23 to 0.48)	0.33 (–0.25 to 0.64)
Liver	0.63	0.92	0.00 (0.00, 1.25)	0.54	0.73	0.18 (0, 1)	0.09 (–0.22 to 0.41)	–0.18 (–0.44 to 0.17)
Cardiovascular	2.39	1.22	2.17 (1.25, 4.00)	1.98	1.18	1.59 (1, 2.95)	0.41 (–0.05 to 0.86)	0.57 (–0.02 to 1.50)
Renal	1.65	1.57	1.45 (0.00, 3.20)	1.13	1.39	0.50 (0.09, 1.57)	0.52 (–0.05 to 1.08)	0.95 (–0.44 to 2.12)
Total	7.42	3.86	6.25 (4.43, 10.5)	6.08	3.74	5.36 (3.69, 7.21)	1.34 (–0.11 to 2.77)	0.89 (–0.27 to 3.00)

LQ, lower quartile; UQ, upper quartile.

a Calculated using bootstrapping.

b Presented on the square root scale as there is no suitable back-transform to an interpretable scale.

Central venous oxygen saturation

Patients were categorised according to ScvO₂ measured at baseline as low (< 70%, 86 patients), normal (70–85%, 233 patients) and high (> 85%, 21 patients).

Tables 29–31 show the mean total SOFA scores for the low, normal and high ScvO₂ groups, respectively.

TABLE 29 Mean total SOFA scores between randomisation and ICU discharge: low ScvO₂

SOFA component	Treatment group						Absolute difference (levosimendan – placebo)	
	Levosimendan			Placebo			Mean (95% CI) ^a	Median (95% CI) ^b
	Mean	SD	Median (LQ, UQ)	Mean	SD	Median (LQ, UQ)		
Respiration	1.42	1.32	1.25 (0.00, 2.42)	1.65	1.45	1.55 (0.03, 3.00)	–0.23 (–0.80 to 0.33)	–0.30 (–1.29 to 0.80)
Coagulation	0.95	1.18	0.50 (0.00, 1.86)	0.75	0.93	0.36 (0.00, 1.00)	0.20 (–0.23 to 0.63)	0.14 (–0.65 to 0.67)
Liver	0.49	0.81	0.00 (0.00, 0.67)	0.67	0.83	0.09 (0.00, 1.00)	–0.18 (–0.51 to 0.16)	–0.09 (–0.95 to 0.39)
Cardiovascular	2.02	1.12	1.90 (1.05, 2.83)	2.33	1.37	1.81 (1.35, 4.00)	–0.31 (–0.83 to 0.21)	0.09 (–1.67 to 0.69)
Renal	1.47	1.43	1.00 (0.09, 2.49)	1.75	1.55	1.92 (0.05, 3.07)	–0.28 (–0.89 to 0.33)	–0.92 (–1.67 to 0.86)
Total	6.34	3.55	5.44 (3.78, 7.64)	7.15	4.76	5.67 (3.34, 11.94)	–0.80 (–2.53 to 0.93)	–0.23 (–3.53 to 1.47)

LQ, lower quartile; UQ, upper quartile.

a Calculated using bootstrapping.

b Presented on the square root scale as there is no suitable back-transform to an interpretable scale.

TABLE 30 Mean total SOFA scores between randomisation and ICU discharge: normal ScvO₂

SOFA component	Treatment group						Absolute difference (levosimendan – placebo)	
	Levosimendan			Placebo			Mean (95% CI) ^a	Median (95% CI) ^a
	Mean	SD	Median (LQ, UQ)	Mean	SD	Median (LQ, UQ)		
Respiration	1.71	1.07	1.56 (1.00, 2.64)	1.56	1.00	1.52 (0.78, 2.2)	0.15 (–0.12 to 0.41)	0.04 (–0.33 to 0.58)
Coagulation	0.66	0.89	0.14 (0.00, 1.00)	0.68	0.98	0.29 (0.00, 1.00)	–0.02 (–0.26 to 0.22)	–0.15 (–0.31 to 0.27)
Liver	0.54	0.87	0.00 (0, 1)	0.45	0.80	0.00 (0, 0.56)	0.09 (–0.12 to 0.30)	0.00 (–0.12 to 0.07)
Cardiovascular	2.21	1.15	2.00 (1.28, 3.24)	1.94	1.10	1.61 (1.07, 2.81)	0.27 (–0.02 to 0.56)	0.39 (–0.01 to 0.71)
Renal	1.42	1.53	0.75 (0, 3)	1.17	1.24	0.79 (0.05, 1.97)	0.26 (–0.10 to 0.62)	–0.04 (–0.66 to 0.75)
Total	6.53	3.94	5.67 (3.67, 8.56)	5.79	3.62	4.86 (3.33, 7.04)	0.74 (–0.22 to 1.71)	0.81 (–0.55 to 1.69)

LQ, lower quartile values; UQ, upper quartile values.
 a Calculated using bootstrapping.

TABLE 31 Mean total SOFA scores between randomisation and ICU discharge: high ScvO₂

SOFA component	Treatment group						Absolute difference (levosimendan – placebo)	
	Levosimendan			Placebo			Mean (95% CI) ^a	Median (95% CI) ^a
	Mean	SD	Median (LQ, UQ)	Mean	SD	Median (LQ, UQ)		
Respiration	1.91	1.26	2.00 (1.33, 3)	2.23	0.94	2.12 (1.69, 2.68)	–0.32 (–1.27 to 0.57)	–0.12 (–1.90 to 1.23)
Coagulation	0.61	0.80	0.00 (0.00, 1.00)	0.89	1.35	0.13 (0.00, 1.19)	–0.28 (–1.19 to 0.57)	–0.13 (–1.29 to 1.00)
Liver	0.22	0.52	0.00 (0.00, 0.00)	0.47	0.84	0.00 (0.00, 0.58)	–0.25 (–0.83 to 0.29)	0.00 (–0.72 to 0.17)
Cardiovascular	2.18	1.26	2.11 (1.57, 2.50)	2.32	1.31	1.67 (1.3, 4.00)	–0.13 (–1.19 to 0.91)	0.44 (–2.18 to 1.50)
Renal	1.12	1.69	0.04 (0.00, 2.43)	1.90	1.70	1.55 (0.40, 3.83)	–0.79 (–2.14 to 0.64)	–1.51 (–3.85 to 1.95)
Total	6.04	3.20	4.93 (3.83, 8.07)	7.81	4.17	6.09 (5.16, 11.12)	–1.77 (–4.78 to 1.19)	–1.16 (–7.00 to 3.18)

LQ, lower quartile values; UQ, upper quartile values.
 a Calculated using bootstrapping.

Lactate

Patients were categorised according to baseline lactate level as normal (≤ 2 mmol/l, 223 patients) and raised (> 2 mmol/l, 282 patients).

Tables 32 and 33 show the mean total SOFA scores for the normal and raised lactate level groups, respectively.

TABLE 32 Mean total SOFA scores between randomisation and ICU discharge: normal lactate level

SOFA component	Treatment group						Absolute difference (levosimendan – placebo)	
	Levosimendan			Placebo			Mean (95% CI) ^a	Median (95% CI) ^a
	Mean	SD	Median (LQ, UQ)	Mean	SD	Median (LQ, UQ)		
Respiration	1.62	1.19	1.73 (0.45, 2.55)	1.49	1.12	1.36 (0.5, 2.19)	0.13 (–0.17 to 0.43)	0.36 (–0.09 to 0.73)
Coagulation	0.50	0.88	0.00 (0.00, 0.65)	0.44	0.81	0.00 (0.00, 0.67)	0.06 (–0.16 to 0.28)	0.00 (–0.12 to 0.06)
Liver	0.32	0.68	0.00 (0.00, 0.19)	0.28	0.64	0.00 (0.00, 0.13)	0.04 (–0.13 to 0.22)	0.00 (0.00 to 0.00)
Cardiovascular	2.12	1.13	1.86 (1.22, 3.00)	1.81	1.07	1.52 (1.00, 2.33)	0.31 (0.02 to 0.60)	0.34 (0.03 to 0.70)
Renal	1.19	1.38	0.54 (0.00, 2.42)	0.91	1.16	0.25 (0.00, 1.53)	0.28 (–0.05 to 0.61)	0.29 (–0.31 to 0.75)
Total	5.76	3.41	5.00 (3.41, 7.25)	4.93	3.05	4.06 (2.99, 6.34)	0.83 (–0.01 to 1.67)	0.94 (0.23 to 1.62)

LQ, lower quartile values; UQ, upper quartile values.
a Calculated using bootstrapping.

TABLE 33 Mean total SOFA score between randomisation and ICU discharge: raised lactate level

SOFA component	Treatment group						Absolute difference (levosimendan – placebo)	
	Levosimendan			Placebo			Mean (95% CI) ^a	Median (95% CI) ^a
	Mean	SD	Median (LQ, UQ)	Mean	SD	Median (LQ, UQ)		
Respiration	1.76	1.19	1.46 (1.00, 2.97)	1.63	1.17	1.60 (0.63, 2.55)	0.13 (–0.14 to 0.41)	–0.14 (–0.58 to 0.47)
Coagulation	0.96	1.15	0.53 (0.00, 1.78)	0.99	1.10	0.64 (0.00, 1.50)	–0.03 (–0.29 to 0.23)	–0.11 (–0.56 to 0.28)
Liver	0.67	0.93	0.11 (0.00, 1.03)	0.58	0.84	0.14 (0.00, 1.00)	0.08 (–0.12 to 0.29)	–0.03 (–0.25 to 0.31)
Cardiovascular	2.40	1.25	2.15 (1.25, 4.00)	2.19	1.25	1.88 (1.05, 3.37)	0.20 (–0.08 to 0.49)	0.28 (–0.23 to 0.92)
Renal	1.71	1.55	1.39 (0.12, 3.08)	1.57	1.46	1.33 (0.13, 2.85)	0.14 (–0.21 to 0.48)	0.06 (–0.63 to 0.95)
Total	7.50	4.23	6.64 (4.06, 10.53)	6.97	4.19	5.86 (4.12, 9.30)	0.52 (–0.45 to 1.50)	0.79 (–0.08 to 1.75)

LQ, lower quartile values; UQ, upper quartile values.
a Calculated using bootstrapping.

Noradrenaline

Patients were categorised into two groups according to baseline noradrenaline level: below the median level ($\leq 0.28 \mu\text{g/kg/minute}$, 259 patients) and above the median level ($> 0.28 \mu\text{g/kg/minute}$, 244 patients).

Tables 34 and 35 show the mean total SOFA scores for the groups with a noradrenaline level below and above the median level, respectively.

TABLE 34 Mean total SOFA score between randomisation and ICU discharge: baseline noradrenaline level below the median

SOFA component	Treatment group						Absolute difference (levosimendan – placebo)	
	Levosimendan			Placebo			Mean (95% CI) ^a	Median (95% CI) ^a
	Mean	SD	Median (LQ, UQ)	Mean	SD	Median (LQ, UQ)		
Respiration	1.41	1.12	1.29 (0.33, 2.44)	1.34	1.12	1.2 (0.35, 2.11)	0.07 (–0.20 to 0.34)	0.09 (–0.25 to 0.46)
Coagulation	0.57	0.99	0.00 (0.00, 0.71)	0.62	0.95	0.10 (0.00, 1.00)	–0.05 (–0.28 to 0.19)	–0.10 (–0.30 to 0.14)
Liver	0.44	0.79	0.00 (0.00, 0.56)	0.39	0.71	0.00 (0.00, 0.43)	0.05 (–0.13 to 0.23)	0.00 (0.00 to 0.00)
Cardiovascular	1.96	1.13	1.67 (1.00, 3.00)	1.80	1.13	1.50 (1.00, 2.50)	0.16 (–0.11 to 0.43)	0.17 (–0.20 to 0.50)
Renal	1.13	1.38	0.31 (0.00, 2.40)	1.01	1.17	0.57 (0.00, 1.77)	0.12 (–0.19 to 0.43)	–0.26 (–0.59 to 0.33)
Total	5.51	3.42	4.57 (3.25, 7.00)	5.16	3.21	4.29 (3.00, 6.65)	0.35 (–0.45 to 1.16)	0.29 (–0.84 to 1.00)

LQ, lower quartile values; UQ, upper quartile values.
 a Calculated using bootstrapping.

TABLE 35 Mean total SOFA score between randomisation and ICU discharge: baseline noradrenaline level above the median

SOFA component	Treatment group						Absolute difference (levosimendan – placebo)	
	Levosimendan			Placebo			Mean (95% CI) ^a	Median (95% CI) ^a
	Mean	SD	Median (LQ, UQ)	Mean	SD	Median (LQ, UQ)		
Respiration	1.94	1.18	1.93 (1.09, 3.00)	1.78	1.15	1.85 (0.77, 2.74)	0.16 (–0.13 to 0.45)	0.08 (–0.31 to 0.52)
Coagulation	0.90	1.08	0.53 (0.00, 1.73)	0.89	1.06	0.54 (0.00, 1.19)	0.01 (–0.26 to 0.27)	–0.01 (–0.56 to 0.36)
Liver	0.55	0.85	0.00 (0.00, 0.83)	0.52	0.84	0.00 (0.00, 0.89)	0.03 (–0.18 to 0.24)	0.00 (–0.16 to 0.17)
Cardiovascular	2.52	1.19	2.34 (1.43, 4.00)	2.23	1.22	1.83 (1.15, 3.66)	0.30 (–0.01 to 0.59)	0.51 (–0.02 to 1.00)
Renal	1.72	1.52	1.38 (0.16, 3.20)	1.57	1.52	1.15 (0.07, 3.00)	0.15 (–0.22 to 0.53)	0.23 (–0.62 to 0.99)
Total	7.64	4.04	6.48 (4.29, 10.65)	6.99	4.29	5.63 (3.78, 9.88)	0.65 (–0.39 to 1.68)	0.85 (–0.04 to 2.40)

LQ, lower quartile values; UQ, upper quartile values.
 a Calculated using bootstrapping.

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