**Levosimendan in septic shock in patients with biochemical evidence of cardiac dysfunction – a subgroup analysis of the LeoPARDS randomised trial.**

## Methods

*Inclusion Criteria*

The target population was adult patients (≥18 years) who required vasopressor support for the management of sepsis despite fluid resuscitation. Inclusion criteria used the internationally established consensus definitions of sepsis at that time [1].

• Fulfil 2/4 of the criteria of the systemic inflammatory response syndrome (SIRS) due to known or suspected infection within the previous 24 hours.

The SIRS criteria were:

(1) fever (>38 °C) or hypothermia (< 36 °C),

(2) tachycardia (heart rate > 90 beats per minute),

(3) tachypnoea (respiratory rate > 20 breaths per minute or $PaCO_2$ < 4.3 kPa) or need for mechanical ventilation,

(4) abnormal leukocyte count (> 12,000 cells/mm3, < 4000 cells/mm3, or > 10% immature [band] forms).

• Hypotension, despite adequate intravenous fluid resuscitation, requiring treatment with a vasopressor infusion (e.g. noradrenaline / adrenaline / vasopressin analogue) for at least four hours and still having an ongoing vasopressor requirement at the time of randomisation. Adequate fluid resuscitation was achieved using repeated fluid challenges.

*Exclusion Criteria*

• More than 24 hours since meeting all 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 (eg Do Not Attempt Resuscitation or not for ventilation/ dialysis)

• Known or estimated weight >135kg

• 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 within 30 days or currently in another interventional trial that might interact with the study drug.

*Missing data*

The calculation of the mean daily SOFA score requires a total SOFA score for each day the patient is in ICU, with the total SOFA being the sum of five components.

The proportion of daily scores that were missing was 6.2% overall. By component, 2.3% of scores were missing for the cardiovascular system, 3.9% for respiratory, 5.1% for renal, 12.8% for hepatic and 6.8% for coagulation. The clinical expectation was that measurements may not be taken if there was no change. Therefore, we imputed values using last observation carried forward as planned. The primary outcome was imputed as follows. Where there were only one or two consecutive days missing, or where the missing data occurred at the end of

the ICU admission, SOFA scores were imputed using last observation carried forward. If the first day was missing the value from day two was taken, and if these were both missing the baseline value was taken. Where there were three or more days missing, the average value of the last available and next available observation was used as the imputed value.

*Modelling biomarker trajectories*

Bayesian hierarchical regression models were used to evaluate whether biomarker trajectories differed for patients receiving levosimendan compared to those in the placebo group. Assuming a biomarker B follows a log-normal distribution, and assuming a linear change over time, we specified the model as follows:

$$\log B_{it} \sim \mathrm{N}\left(\mu_{it}, \sigma_B^2\right)$$

$$\boldsymbol{\mu_{it}} = \boldsymbol{\alpha} + \boldsymbol{\beta_1} \log B b_i + \beta_2 trt_i + \beta_3 t + \beta_4 trt_i \times t + U_i$$

The following vague prior distributions were used:

$$\alpha, \beta_1, \ldots, \beta_4 \sim \mathrm{N}(0, 100000)$$

$$U_i \sim \mathrm{N}(0, \sigma_u^2)$$

$$\sigma_u \sim \mathrm{Uniform}(0, 100)$$

Where $B_{it}$ is the biomarker value for patient i at time t (t=0,1,2 corresponding to days 2, 4 and 6 post randomisation respectively), $Bb_i$ is the biomarker value for patient i at baseline, and $trt_i$ is the treatment indicator for patient i (0=placebo, 1=levosimendan), and $U_i$ is a patient-specific random intercept. We presented an estimate of the mean treatment difference at each time point t, given by β2 +β4 ×t with 95% credible intervals, and the

probability that NT-pro BNP reduces faster in Levosimendan patients compared to placebo, given by $Pr(\beta 4 < 0)$.

Any values below/above the limits of detection were treated as missing, but arising from the same distribution as the observed values truncated at the limit of detection. In contrast to using a fixed value falling below/above the limit, this approach acknowledges uncertainty in the values. We used two chains with diffuse starting values and checked convergence using trace plots and the Gelman-Rubin convergence statistic. After convergence, Markov Chain Monte Carlo simulations were run until the effective sample size was around 10,000.

**Results**

*Additional results for biomarker analysis*

Table S1 to S7 show the full results from the hierarchical regression models for cardiovascular biomarkers and inflammatory mediators. Here all results are provided, including models without the treatment x time interaction, along with those for sensitivity analyses adjusting for age and APACHE II score, and for ICU effects. The Deviance Information Criterion (DIC) is included as a measure of model fit. The model with the smallest DIC is considered to fit the data best, though alternative models with a difference of less than 3 should not be ruled out, and differences of less than 7 indicate weak support for the “best” model.

**Table S1: Full results for longitudinal models for troponin I (cTnI)**

| | Main model | No interaction | Adjusting for age and APACHE II | Adjusting for ICU effects |
|---|---|---|---|---|
| Change per day - Levosimendan | 0.87 (0.74,1.01) | 0.80 (0.72,0.89) | 0.87 (0.74,1.01) | 0.87 (0.74,1.01) |
| Change per day - Placebo | 0.75 (0.65,0.86) | | 0.75 (0.64,0.86) | 0.75 (0.64,0.86) |
| Pr(faster reduction in Levosimendan) | 0.082 | | 0.081 | 0.082 |
| Treatment difference on day 2 | 1.12 (0.79,1.55) | 1.26 (0.92,1.67) | 1.14 (0.81,1.58) | 1.12 (0.78,1.55) |
| Treatment difference on day 4 | 1.30 (0.95,1.73) | | 1.33 (0.98,1.77) | 1.30 (0.95,1.73) |
| Treatment difference on day 6 | 1.52 (1.01,2.19) | | 1.56 (1.03,2.25) | 1.52 (1.01,2.21) |
| *Change per:* | | | | |
| 10% increase in baseline cTnI | 1.03 (1.02,1.04) | 1.03 (1.02,1.04) | 1.03 (1.02,1.03) | 1.03 (1.02,1.04) |
| 1 year increase in age | | | 1.01 (1.00,1.02) | |
| Unit increase in APACHE II score | | | 1.05 (1.03,1.07) | |
| *Random effects variance:* | | | | |
| Patient intercept | 1.70 (1.37,2.07) | 1.69 (1.37,2.06) | 1.63 (1.31,1.98) | 1.69 (1.36,2.05) |
| ICU intercept | | | | 0.03 (0.00,0.13) |
| DIC | 4104.3 | 4106.0 | 4094.7 | 4104.9 |

**Table S2: Full results for longitudinal models for NT-pro BNP**

| | Main model | No interaction | Adjusting for age and APACHE II | Adjusting for ICU effects |
|---|---|---|---|---|
| Change per day - Levosimendan | 1.09 (1.00,1.19) | 1.02 (0.96,1.09) | 1.09 (1.00,1.19) | 1.09 (1.00,1.19) |
| Change per day - Placebo | 0.97 (0.90,1.05) | | 0.97 (0.90,1.05) | 0.97 (0.90,1.06) |
| Pr(faster reduction in Levosimendan) | 0.032 | | 0.032 | 0.035 |
| Treatment difference on day 2 | 1.00 (0.84,1.19) | 1.10 (0.94,1.27) | 1.01 (0.84,1.20) | 1.00 (0.84,1.19) |
| Treatment difference on day 4 | 1.12 (0.96,1.30) | | 1.13 (0.97,1.31) | 1.12 (0.96,1.30) |
| Treatment difference on day 6 | 1.26 (1.02,1.54) | | 1.27 (1.02,1.56) | 1.26 (1.02,1.54) |
| *Change per:* | | | | |
| 10% increase in baseline NT-pro BNP | 1.07 (1.06,1.07) | 1.07 (1.06,1.07) | 1.07 (1.06,1.07) | 1.07 (1.06,1.07) |
| 1 year increase in age | | | 1.00 (0.99,1.01) | |
| Unit increase in APACHE II score | | | 1.01 (1.00,1.02) | |
| *Random effects variance:* | | | | |
| Patient intercept | 0.36 (0.27,0.46) | 0.36 (0.27,0.46) | 0.36 (0.27,0.46) | 0.35 (0.26,0.45) |
| ICU intercept | | | | 0.01 (0.00,0.05) |
| DIC | 2723.1 | 2725.4 | 2720.8 | 2723.3 |

**Table S3: Full results for longitudinal models for CCL2**

| | Main model | No interaction | Adjusting for age and APACHE II | Adjusting for ICU effects |
|---|---|---|---|---|
| Change per day - Levosimendan | 0.78 (0.73,0.83) | 0.74 (0.71,0.77) | 0.78 (0.73,0.83) | 0.76 (0.71,0.81) |
| Change per day - Placebo | 0.71 (0.66,0.75) | | 0.71 (0.66,0.75) | 0.71 (0.66,0.75) |
| Pr(faster reduction in Levosimendan) | 0.019 | | 0.018 | 0.079 |
| Treatment difference on day 2 | 0.89 (0.78,1.02) | 0.96 (0.86,1.07) | 0.89 (0.78,1.02) | 0.86 (0.76,0.97) |
| Treatment difference on day 4 | 0.98 (0.87,1.10) | | 0.98 (0.88,1.10) | 0.92 (0.84,1.00) |
| Treatment difference on day 6 | 1.08 (0.92,1.26) | | 1.08 (0.92,1.26) | 0.98 (0.85,1.13) |
| *Change per:* | | | | |
| 10% increase in baseline CCL2 | 1.05 (1.04,1.06) | 1.05 (1.04,1.06) | 1.05 (1.04,1.05) | 1.05 (1.04,1.06) |
| 1 year increase in age | | | 1.00 (0.99,1.00) | |
| Unit increase in APACHE II score | | | 1.01 (1.00,1.02) | |
| *Random effects variance:* | | | | |
| Patient intercept | 0.22 (0.17,0.27) | 0.22 (0.17,0.27) | 0.21 (0.16,0.27) | 0.19 (0.14,0.24) |
| ICU intercept | | | | 0.02 (0.00,0.06) |
| DIC | 2186.4 | 2191.9 | 2182.0 | 2204.0 |

**Table S4: Full results for longitudinal models for IL6**

| | Main model | No interaction | Adjusting for age and APACHE II | Adjusting for ICU effects |
|---|---|---|---|---|
| Change per day - Levosimendan | 0.50 (0.44,0.56) | 0.50 (0.46,0.54) | 0.50 (0.44,0.56) | 0.50 (0.44,0.56) |
| Change per day - Placebo | 0.50 (0.45,0.56) | | 0.50 (0.45,0.56) | 0.50 (0.45,0.56) |
| Pr(faster reduction in Levosimendan) | 0.536 | | 0.532 | 0.552 |
| Treatment difference on day 2 | 1.00 (0.79,1.25) | 0.99 (0.82,1.20) | 1.01 (0.79,1.26) | 1.01 (0.80,1.25) |
| Treatment difference on day 4 | 0.99 (0.81,1.20) | | 1.00 (0.82,1.20) | 1.00 (0.83,1.19) |
| Treatment difference on day 6 | 0.99 (0.74,1.29) | | 1.00 (0.75,1.30) | 0.99 (0.75,1.28) |
| *Change per:* | | | | |
| 10% increase in baseline IL6 | 1.04 (1.03,1.04) | 1.04 (1.03,1.04) | 1.04 (1.03,1.04) | 1.04 (1.03,1.04) |
| 1 year increase in age | | | 1.00 (0.99,1.01) | |
| Unit increase in APACHE II score | | | 1.02 (1.00,1.03) | |
| *Random effects variance:* | | | | |
| Patient intercept | 0.54 (0.39,0.70) | 0.54 (0.39,0.70) | 0.53 (0.39,0.70) | 0.44 (0.30,0.60) |
| ICU intercept | | | | 0.10 (0.03,0.21) |
| DIC | 3462.2 | 3459.7 | 3459.3 | 3452.3 |

**Table S5: Full results for longitudinal models for IL8**

| | Main model | No interaction | Adjusting for age and APACHE II | Adjusting for ICU effects |
|---|---|---|---|---|
| Change per day - Levosimendan | 0.85 (0.80,0.91) | 0.82 (0.79,0.86) | 0.85 (0.80,0.91) | 0.85 (0.80,0.91) |
| Change per day - Placebo | 0.80 (0.75,0.84) | | 0.79 (0.75,0.84) | 0.79 (0.75,0.84) |
| Pr(faster reduction in Levosimendan) | 0.046 | | 0.048 | 0.048 |
| Treatment difference on day 2 | 1.00 (0.85,1.17) | 1.06 (0.91,1.22) | 1.00 (0.85,1.17) | 1.00 (0.86,1.17) |
| Treatment difference on day 4 | 1.08 (0.93,1.24) | | 1.08 (0.93,1.25) | 1.08 (0.94,1.24) |
| Treatment difference on day 6 | 1.16 (0.97,1.38) | | 1.16 (0.97,1.38) | 1.16 (0.97,1.38) |
| *Change per:* | | | | |
| 10% increase in baseline IL8 | 1.05 (1.05,1.06) | 1.05 (1.05,1.06) | 1.05 (1.05,1.06) | 1.06 (1.05,1.06) |
| 1 year increase in age | | | 1.00 (0.99,1.00) | |
| Unit increase in APACHE II score | | | 1.02 (1.01,1.03) | |
| *Random effects variance:* | | | | |
| Patient intercept | 0.48 (0.40,0.58) | 0.48 (0.40,0.57) | 0.47 (0.39,0.56) | 0.44 (0.36,0.52) |
| ICU intercept | | | | 0.05 (0.01,0.11) |
| DIC | 2112.6 | 2115.7 | 2109.0 | 2108.9 |

**Table S6: Full results for longitudinal models for IL10**

| | Main model | No interaction | Adjusting for age and APACHE II | Adjusting for ICU effects |
|---|---|---|---|---|
| Change per day - Levosimendan | 0.68 (0.63,0.73) | 0.69 (0.65,0.72) | 0.68 (0.63,0.73) | 0.68 (0.63,0.73) |
| Change per day - Placebo | 0.69 (0.65,0.74) | | 0.69 (0.65,0.74) | 0.69 (0.65,0.74) |
| Pr(faster reduction in Levosimendan) | 0.662 | | 0.644 | 0.671 |
| Treatment difference on day 2 | 1.06 (0.90,1.25) | 1.05 (0.91,1.20) | 1.07 (0.91,1.25) | 1.07 (0.91,1.25) |
| Treatment difference on day 4 | 1.04 (0.90,1.19) | | 1.05 (0.91,1.20) | 1.05 (0.91,1.20) |
| Treatment difference on day 6 | 1.02 (0.85,1.23) | | 1.03 (0.85,1.23) | 1.02 (0.85,1.23) |
| *Change per:* | | | | |
| 10% increase in baseline IL10 | 1.04 (1.04,1.05) | 1.04 (1.04,1.05) | 1.04 (1.04,1.05) | 1.04 (1.04,1.05) |
| 1 year increase in age | | | 1.00 (1.00,1.01) | |
| Unit increase in APACHE II score | | | 1.02 (1.01,1.03) | |
| *Random effects variance:* | | | | |
| Patient intercept | 0.38 (0.30,0.47) | 0.38 (0.30,0.47) | 0.37 (0.29,0.45) | 0.36 (0.28,0.44) |
| ICU intercept | | | | 0.02 (0.00,0.06) |
| DIC | 2450.2 | 2447.0 | 2443.8 | 2449.4 |

**Table S7: Full results for longitudinal models for sTNFr1**

| | Main model | No interaction | Adjusting for age and APACHE II | Adjusting for ICU effects |
|---|---|---|---|---|
| Change per day - Levosimendan | 0.90 (0.86,0.93) | 0.90 (0.87,0.92) | 0.90 (0.86,0.93) | 0.89 (0.86,0.93) |
| Change per day - Placebo | 0.90 (0.86,0.93) | | 0.89 (0.86,0.93) | 0.90 (0.86,0.93) |
| Pr(faster reduction in Levosimendan) | 0.500 | | 0.492 | 0.513 |
| Treatment difference on day 2 | 1.02 (0.93,1.12) | 1.02 (0.94,1.11) | 1.02 (0.93,1.12) | 1.02 (0.93,1.12) |
| Treatment difference on day 4 | 1.02 (0.94,1.11) | | 1.02 (0.94,1.11) | 1.02 (0.94,1.11) |
| Treatment difference on day 6 | 1.02 (0.92,1.13) | | 1.02 (0.92,1.14) | 1.02 (0.92,1.13) |
| *Change per:* | | | | |
| 10% increase in baseline sTNFr1 | 1.08 (1.07,1.08) | 1.08 (1.07,1.08) | 1.08 (1.07,1.08) | 1.08 (1.07,1.08) |
| 1 year increase in age | | | 1.00 (1.00,1.00) | |
| Unit increase in APACHE II score | | | 1.01 (1.00,1.01) | |
| *Random effects variance:* | | | | |
| Patient intercept | 0.14 (0.11,0.17) | 0.14 (0.12,0.17) | 0.14 (0.11,0.17) | 0.13 (0.11,0.16) |
| ICU intercept | | | | 0.17 (0.00,0.03) |
| DIC | 1174.1 | 1171.6 | 1172.1 | 1173.1 |

**Figure S1** Boxplot of troponin I values by timepoint of sampling comparing those receiving levosimendan (red boxes) or placebo (green boxes). Day 1 was prior to randomisation on day of inclusion and day 2 after 24 hours of treatment. The number of patients sampled on each day on intensive care are given below the figure.

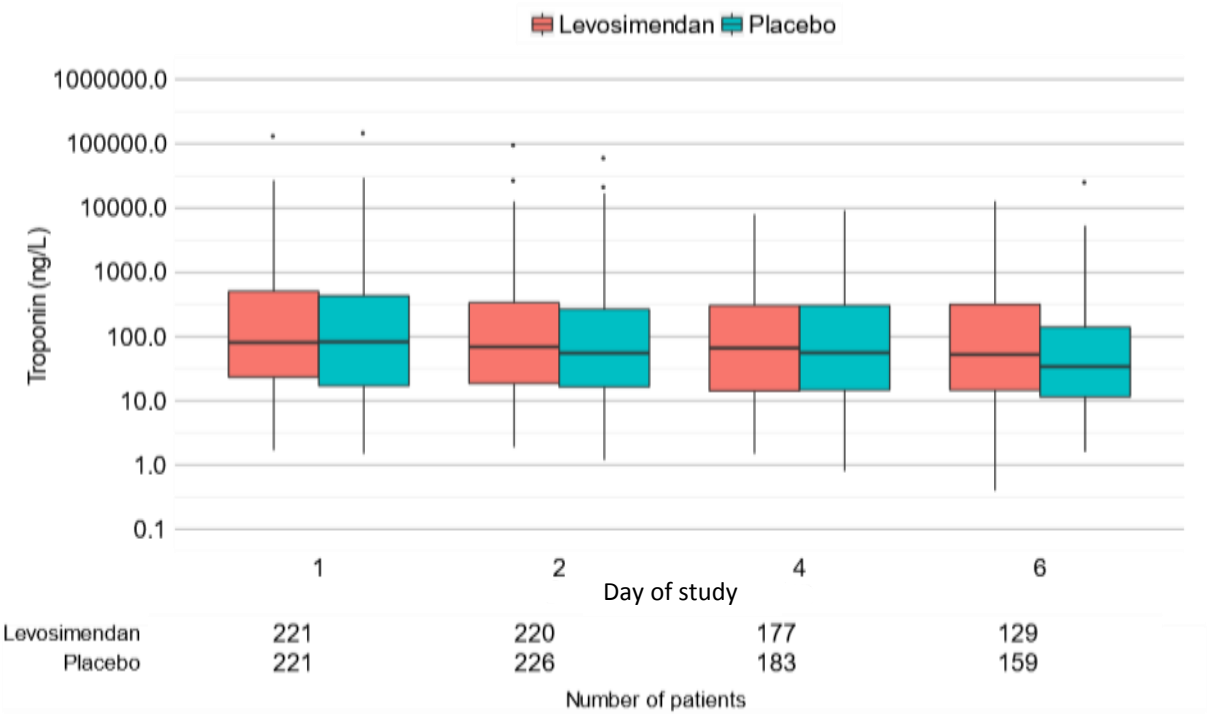


**Figure S2** Boxplot of NT-proBNP values by timepoint of sampling comparing those receiving levosimendan (red boxes) or placebo (green boxes). Day 1 was prior to randomisation on day of inclusion and day 2 after 24 hours of treatment. The number of patients sampled on each day on intensive care are given below the figure.

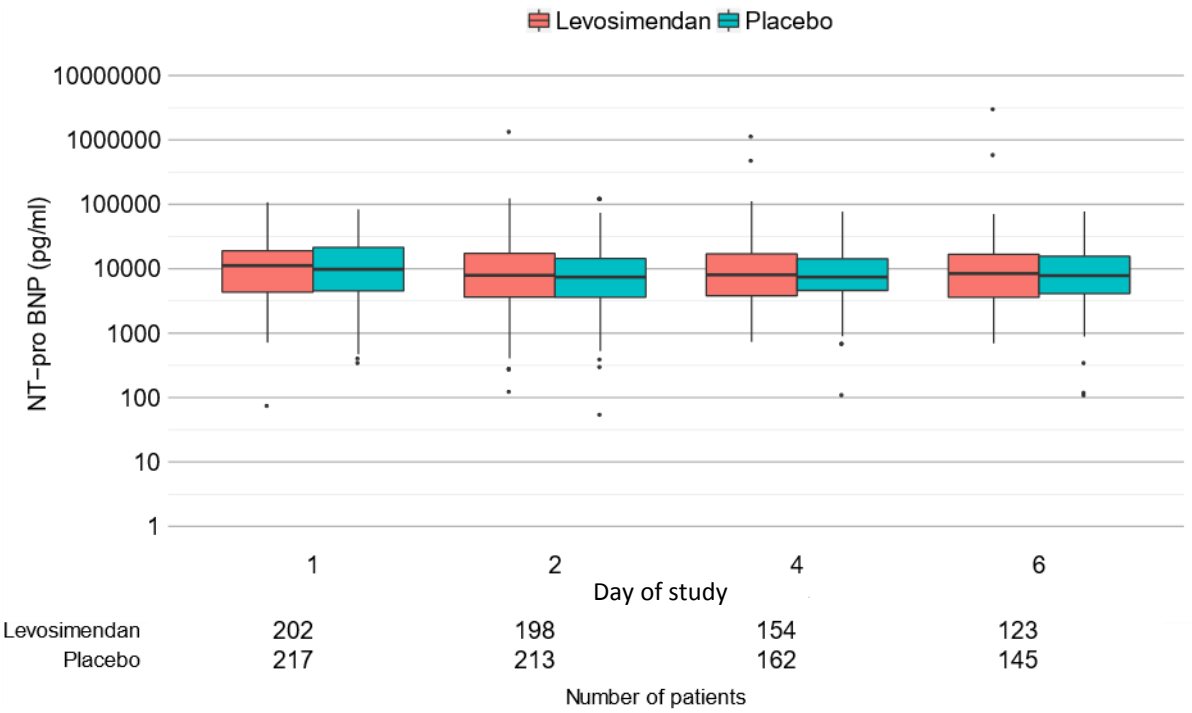
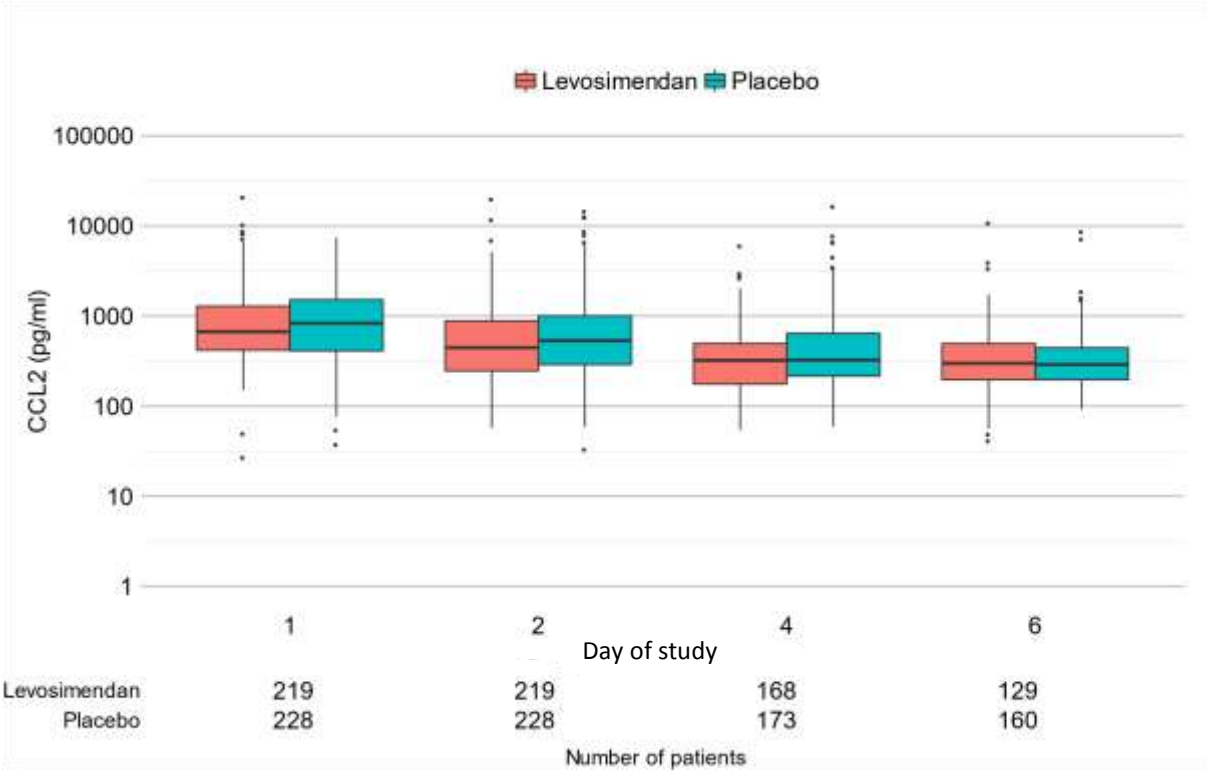
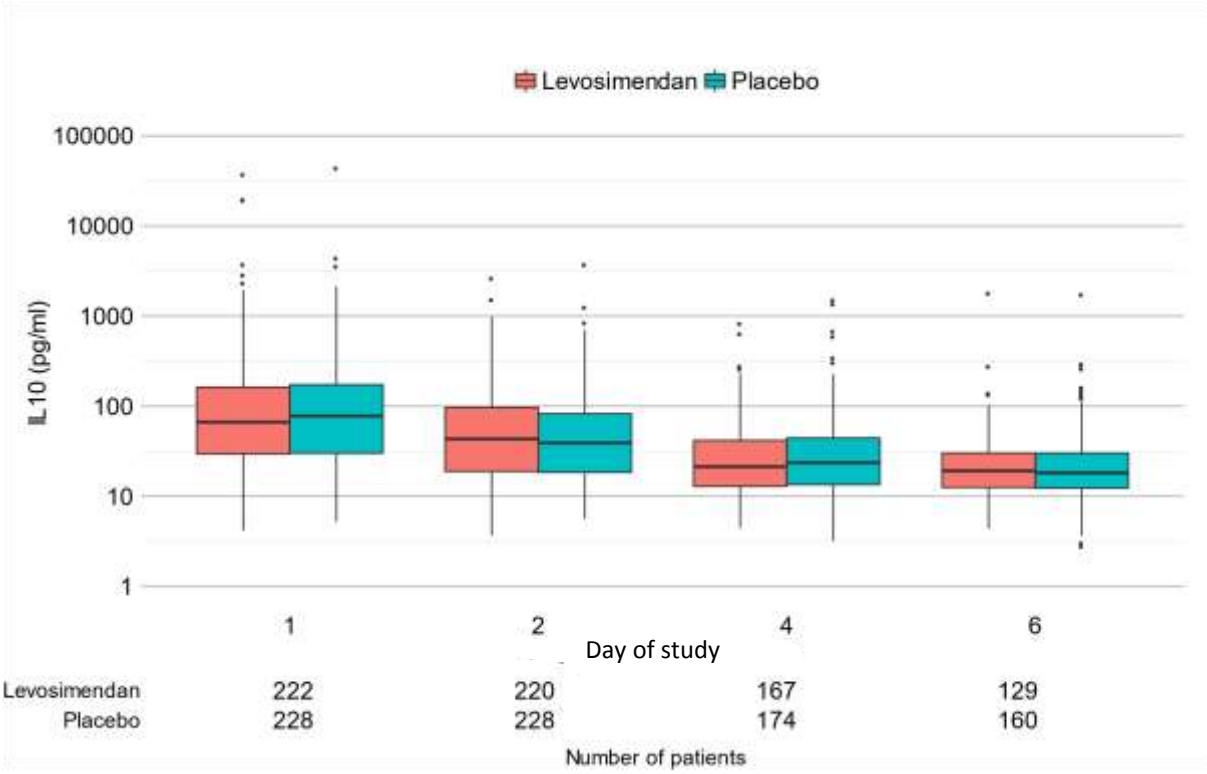


**Figure S3** Boxplots of **a** CCL2 **b** IL10 **c** IL6 **d** IL8 and **e** sTNFr1 measurements by timepoint of sampling comparing those receiving levosimendan (red boxes) or placebo (green boxes). Day 1 was prior to randomisation on day of inclusion and day 2 after 24 hours of treatment. The number of patients sampled on each day on intensive care are given below the figure.

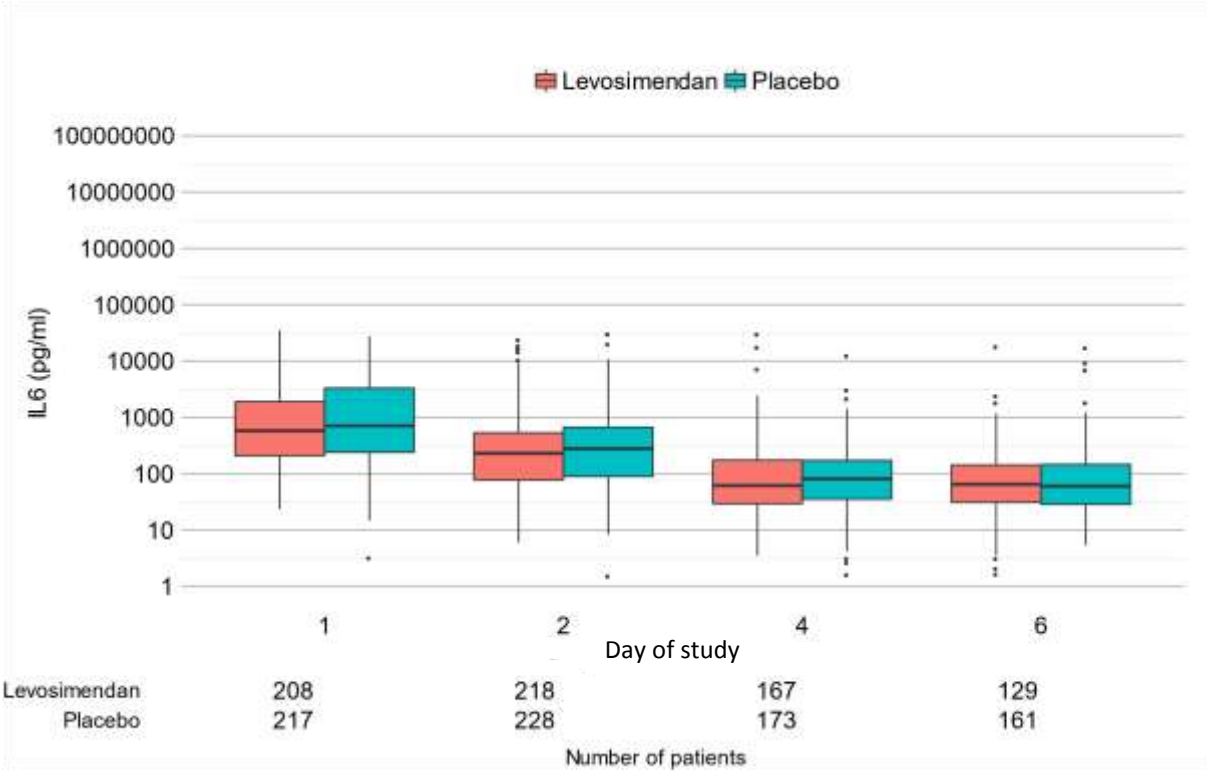
**a**



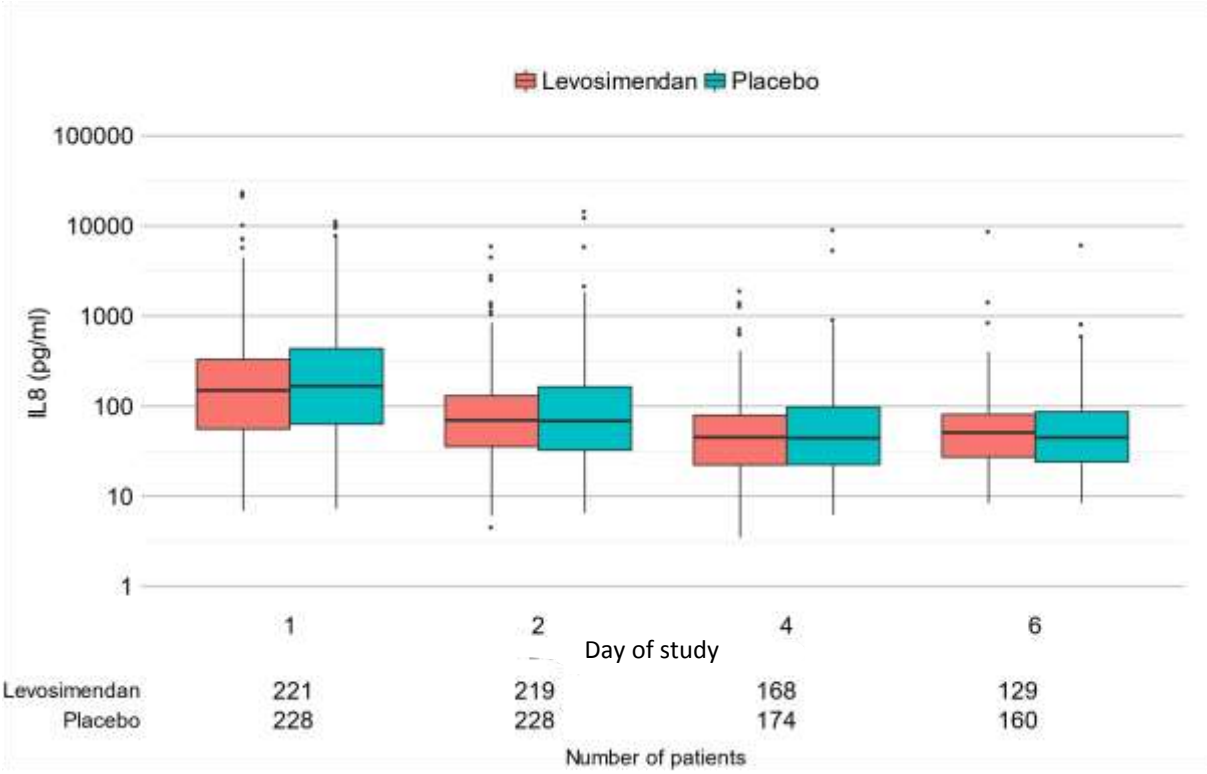
**b**


Levosimendan
Placebo
100000
10000
1000
100
10
1
IL10 (pg/ml)
1
2
4
6
Day of study
Levosimendan 222 220 167 129
Placebo 228 228 174 160
Number of patients

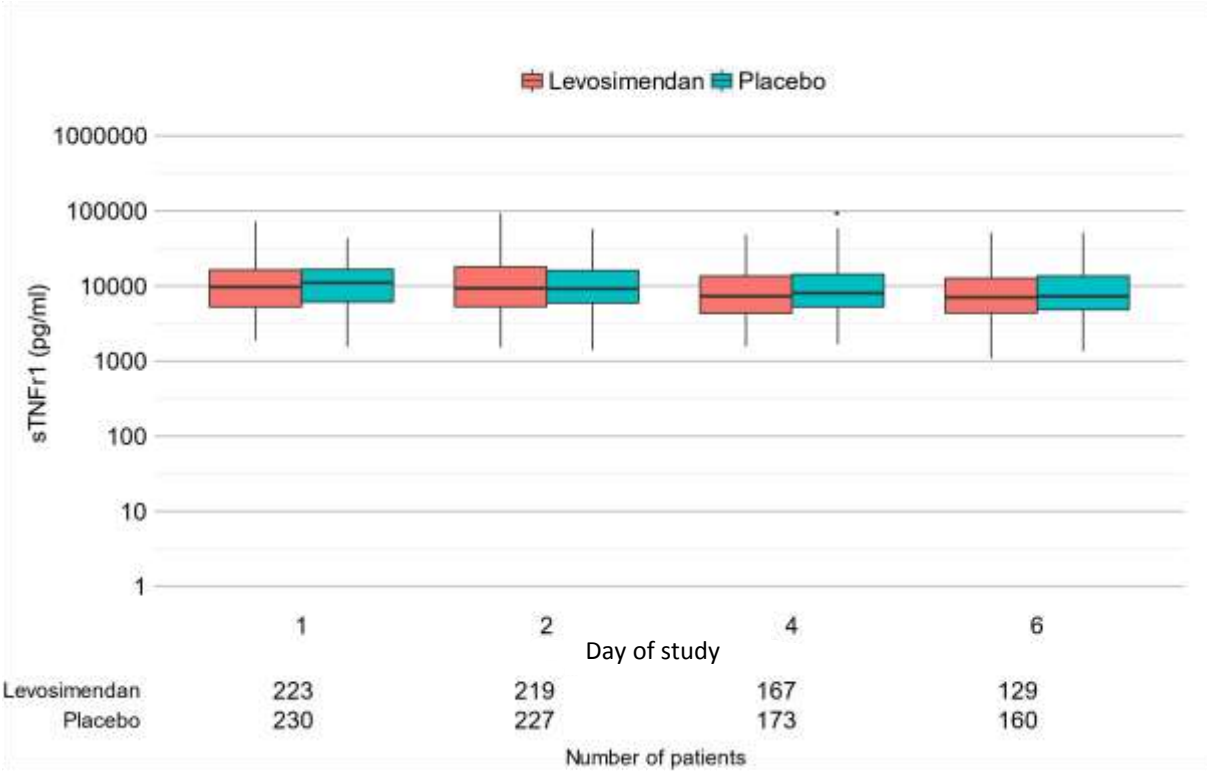
**c**


Levosimendan
Placebo
100000000
10000000
1000000
100000
10000
1000
100
10
1
IL6 (pg/ml)
1
2
4
6
Day of study
Levosimendan 208 218 167 129
Placebo 217 228 173 161
Number of patients

**d**


Levosimendan
Placebo
100000
10000
1000
100
10
1
IL8 (pg/ml)
1
2
4
6
Day of study
Levosimendan 221 219 168 129
Placebo 228 228 174 160
Number of patients

**e**


Levosimendan
Placebo
1000000
100000
10000
1000
100
10
1
sTNFr1 (pg/ml)
1
2
4
6
Day of study
Levosimendan 223 219 167 129
Placebo 230 227 173 160
Number of patients

**References**

1. Members of the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Committee (1992) American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Crit Care Med 20:864–874. https://doi.org/10.1097/00003246-199206000-00025