

Supplementary Appendix

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

Supplement to: Russell JA, Walley KR, Singer J, et al. Vasopressin versus norepinephrine infusion in patients with septic shock. *N Engl J Med* 2008;358:877-87.

Online Supplement

Methods

Septic shock definition

SIRS criteria were:

- (1) fever ($>38^{\circ}\text{C}$) or hypothermia ($<36^{\circ}\text{C}$),
- (2) tachycardia (heart rate >90 beats per minute),
- (3) tachypnea (respiratory rate >20 breaths per minute or $\text{PaCO}_2 < 32$ mmHg) or need for mechanical ventilation,
- (4) abnormal leukocyte count ($>12,000$ cells/ mm^3 , <4000 cells/ mm^3 , or $>10\%$ immature [band] forms).

Known or suspected infection was defined by clinical suspicion of infection (microbiology cultures were positive or pending and the patient was being treated using antibiotics).

Hypotension was defined by: systolic blood pressure (SBP) less than 90 mmHg or decrease in SBP by at least 40 mmHg for more than one hour while central venous pressures (CVP) remained adequate (≥ 12 mmHg) or at least 500 mL of saline was infused or if vasopressors were infused to maintain blood pressure.

Vasopressor requirement. Norepinephrine equivalent dose was calculated as [norepinephrine ($\mu\text{g}/\text{min}$)] + [dopamine ($\mu\text{g}/\text{kg}/\text{min}$) $\div 2$] + [epinephrine ($\mu\text{g}/\text{min}$)] + [phenylephrine ($\mu\text{g}/\text{min}$) $\div 10$] after the study of Patel *et al.*¹⁰ Patients needed to have received ≥ 5 $\mu\text{g}/\text{min}$ of norepinephrine or equivalent for at least six consecutive hours in the preceding 24 hours and to have received at

least 5 µg/min within the last hour prior to randomization. We also defined a fast track entry option for patients requiring high vasopressor doses (norepinephrine equivalent ≥ 15 µg/hr for three consecutive hours) who could be randomized at three hours rather than at six hours.

New organ dysfunction was defined as respiratory (ventilated and $\text{PaO}_2/\text{FiO}_2 < 300$ mmHg), renal (urine output < 30 mL/hour or less than 0.5 mL/kg body weight, for at least one hour), coagulation (platelet count $< 80,000/\text{mm}^3$), and neurologic (Glasgow Coma Score < 12 , prior to receiving sedation).

Exclusion criteria

- (1) unstable coronary syndrome (acute myocardial infarction during this episode of shock based on the combination of history, electrocardiogram, and enzyme changes (as defined by investigator),
- (2) greater than 24 hours had elapsed since the patient met entry criteria,
- (3) use of open-label vasopressin for blood pressure support during the current hospital admission,
- (4) malignancy or other irreversible disease or condition for which six-month mortality was estimated to be $\geq 50\%$,
- (5) acute mesenteric ischemia either proven or suspected. A patient could be excluded by the investigator if, in their judgment, the condition was strongly suspected but not proven by conventional criteria or the attending physician had initiated presumptive therapy,
- (6) death anticipated within 12 hours,
- (7) underlying chronic heart disease (NYHA class III or IV) and shock,
- (8) physician and team were not committed to aggressive care,

- (9) severe hyponatremia (serum sodium < 130 mmol/L),
- (10) traumatic brain injury (GCS < 8 prior to onset of sepsis),
- (11) Raynaud's phenomenon, systemic sclerosis or vasospastic diathesis,
- (12) pregnancy (positive serum β -HCG).

Weaning of vasopressors

The weaning of open-label vasopressors was allowed only when target mean arterial pressure (MAP) had been reached during the study drug infusion. Open-label vasopressors were increased only if target MAP was not reached on maximal study drug infusion. When the study drug infusion was at 15 mL/hr, then open-label norepinephrine was weaned by 1-2 μ g/min every 5 - 10 minutes while maintaining target MAP.

Weaning of the study medication was commenced when the target MAP was maintained while off all open-label vasopressors for eight hours. The infusion of study medication was decreased in 2.5 mL decrements every hour while the target MAP was maintained. Time of discontinuation of study drug for at least 12 hours was recorded. In the event of hypertension despite the above weaning protocols (MAP > 85 mmHg), open-label vasopressors and study drug could be weaned more rapidly.

If re-infusion of vasopressors was required once a patient had been weaned from vasopressor support, study drug was recommenced first. In the case of emergency situations that required re-infusion of vasopressors and the study drug was not immediately available, any open-label vasopressor (except open-label vasopressin) could be infused to maintain blood pressure until the study drug was available. An inability to re-infuse study drug within 12 hours (e.g. study pharmacist unavailable) was reported as a protocol violation.

Co-interventions

The effect of co-interventions was minimized because the medical teams were not aware of treatment allocation. Other aspects of managing septic shock and critical illness were at the discretion of the ICU team. However, important co-interventions potentially influencing the primary outcome were recorded.

Plasma vasopressin levels

Blood for measurement of plasma vasopressin levels was collected at six sites (St Paul's, Mount Sinai, Ottawa General, Toronto General, The Alfred and The Mayo Clinic hospitals) at five timepoints; baseline, 6, 24, 72 hours and 7 days. Blood was collected in chilled EDTA bottles, cold centrifuged and the plasma stored at -70°C until analysis. Vasopressin was analyzed after extraction on reversed phase column by double antibody immunoassay (Buhlman Laboratories, Basel, Switzerland) at London Laboratory Services Group, London, Ontario. The sensitivity limit of this assay is 0.39 pmol/L. The precision between runs was 11% at a mean of 1.9 pmol/L, 9.1% at a mean of 10.2 pmol/L, and 8.6% at a mean of 16.5 pmol/L. The within run variations were lower at all levels.

Within the five collecting centers, 107 of 359 (29.8%) patients infused with study drug had vasopressin assays done.

Outcomes

Study personnel visited patients in hospital and contacted patients or healthcare professionals for participants who left the hospital to determine 28-day and 90-day survival. Length of stay in the ICU and hospital was defined as the time spent in ICU or hospital respectively during the index

hospitalization. A patient was considered discharged on the day of transfer home, to alternate care (off active care while in hospital) or to a chronic care facility.

Online Table 1. Brussels organ dysfunction definitions

| Organs | Clinically Significant Organ Dysfunction | | | | |
|---|---|------------------------|---------------------------|-------------------------|-----------------|
| | Normal | Mild | Moderate | Severe | Extreme |
| Cardiovascular (systolic blood pressure, mmHg) | > 90 | ≤ 90 fluid responsive | ≤ 90 not fluid responsive | ≤ 90 pH 7.3 | ≤ 90 pH 7.2 |
| Pulmonary (PaO ₂ /FiO ₂ , mmHg) | > 400 | 400 - 301 | 300 – 201 | 200 – 101 | 100 |
| Neurologic (GCS) | 15 | 14 - 13 | 12 – 10 | 9 – 6 | ≤ 5 |
| Coagulation (platelet count, x10 ³ /mm ³) | > 120 | 120 - 81 | 80 – 51 | 50 – 21 | ≤20 |
| Renal (creatinine, μmol/L [mg/dL]) | <133 [<1.5] | 133 – 175 [1.5-1.9] | 176 – 300 [2.0-3.4] | 301 – 442 [3.5-4.9] | ≥443 [≥5.0] |
| Hepatic (bilirubin, μmol/L [mg/dL]) | < 20 [<1.2] | 20 – 32 [1.2-1.9] | 33 – 99 [2.0-5.9] | 100 – 199 [6.0-11.9] | ≥200 [≥12.0] |

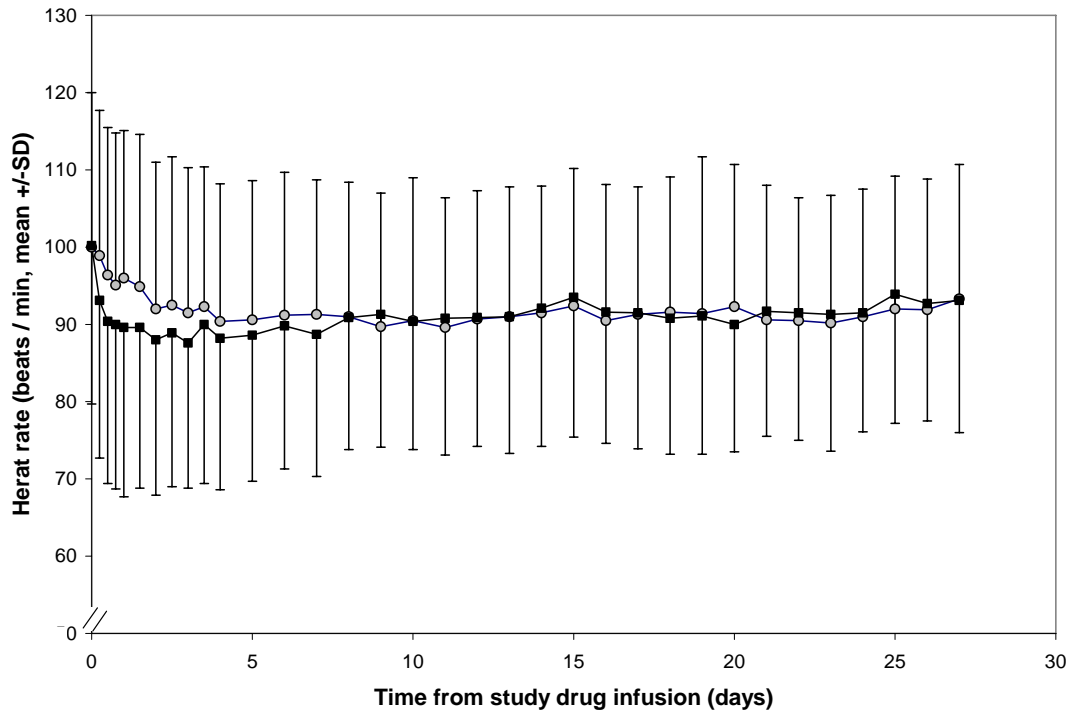
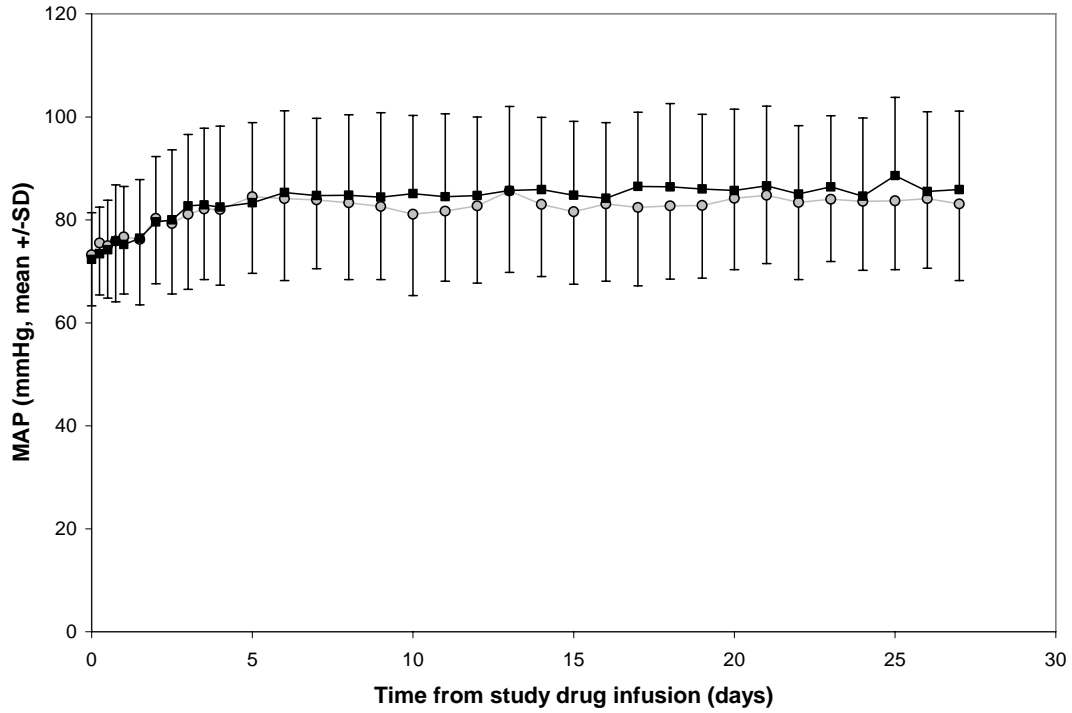
Days alive and free (DAF) calculations. DAF was scored as 1 if the patient was alive and free of organ dysfunction (normal or mild dysfunction). DAF was scored as 0 if the patient had organ dysfunction (moderate, severe, or extreme) or was not alive. A low DAF score indicates more organ dysfunction because a low score indicates fewer days alive and free of organ dysfunction. Each of the 28 days after meeting the inclusion criteria was scored. For any 24-hour period in which there is no measurement of a variable, we carried forward the value from the previous 24-hour period. If any variable was never measured, it was assumed to be normal. Once a patient was discharged home they were considered free of organ or failure.

Protocol / Data Monitoring

To ensure compliance with the study protocol across the multiple sites and verify accurate data collection a process of monitoring case report forms (CRF) was undertaken. Eight trained monitors visited a representative sample of sites to verify important data on the CRF against source documents. This included appropriate ascertainment of consent, accurate application of inclusion and exclusion criteria, compliance with study drug infusion (initiation, maintenance and weaning), 28-day and 90-day mortality, time to hemodynamic stability, protocol violations and any serious adverse events. Geographical and budgetary restrictions prevented 100% monitoring.

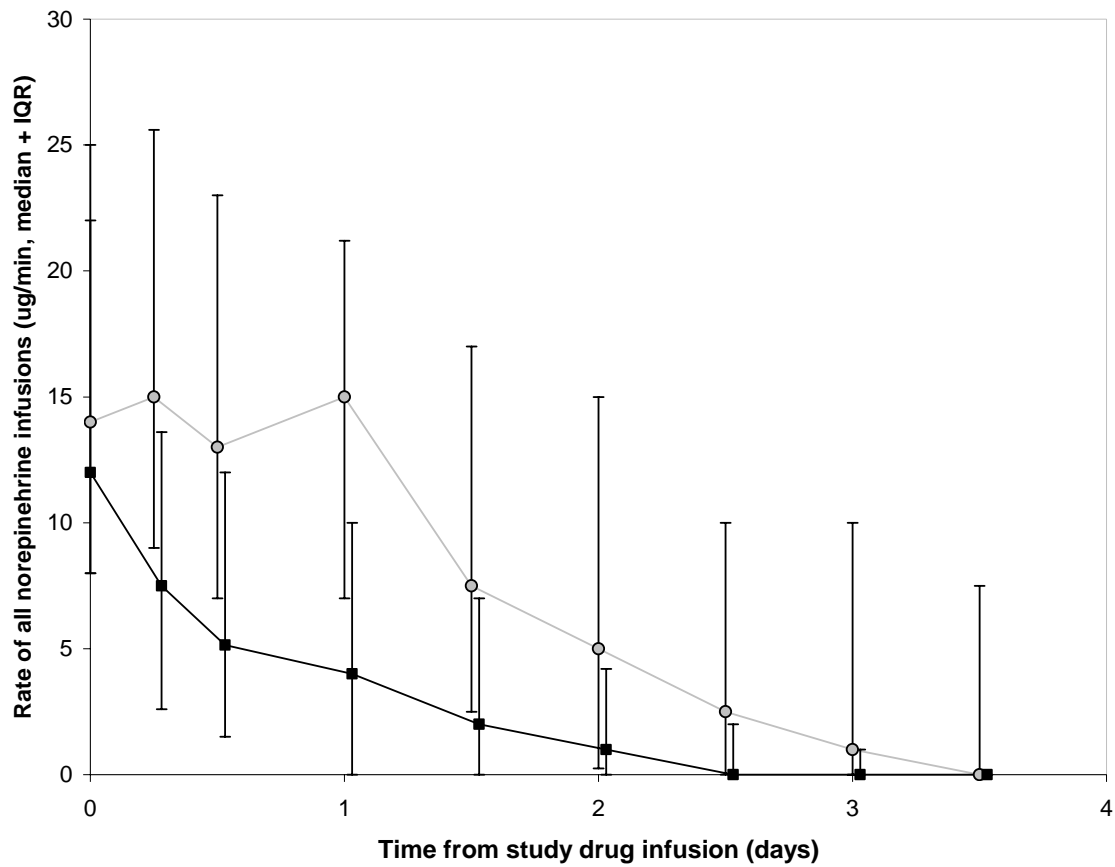
In total 454 (58.3%) charts in 16 sites in Canada and Australia were monitored. In general the quality of data collected on the CRF was very good, there was consistent interpretation of the protocol and CRF, and the determination of outcome was 100% correct. In summary two additional serious adverse events were captured, there were 21 minor variations in the consent documents including incorrect version of consent form used, no witness or investigator signatures, 10 patients were enrolled outside of the 24 hour inclusion window and one patient was found not to have a new additional organ failure on inclusion.

Online supplement results

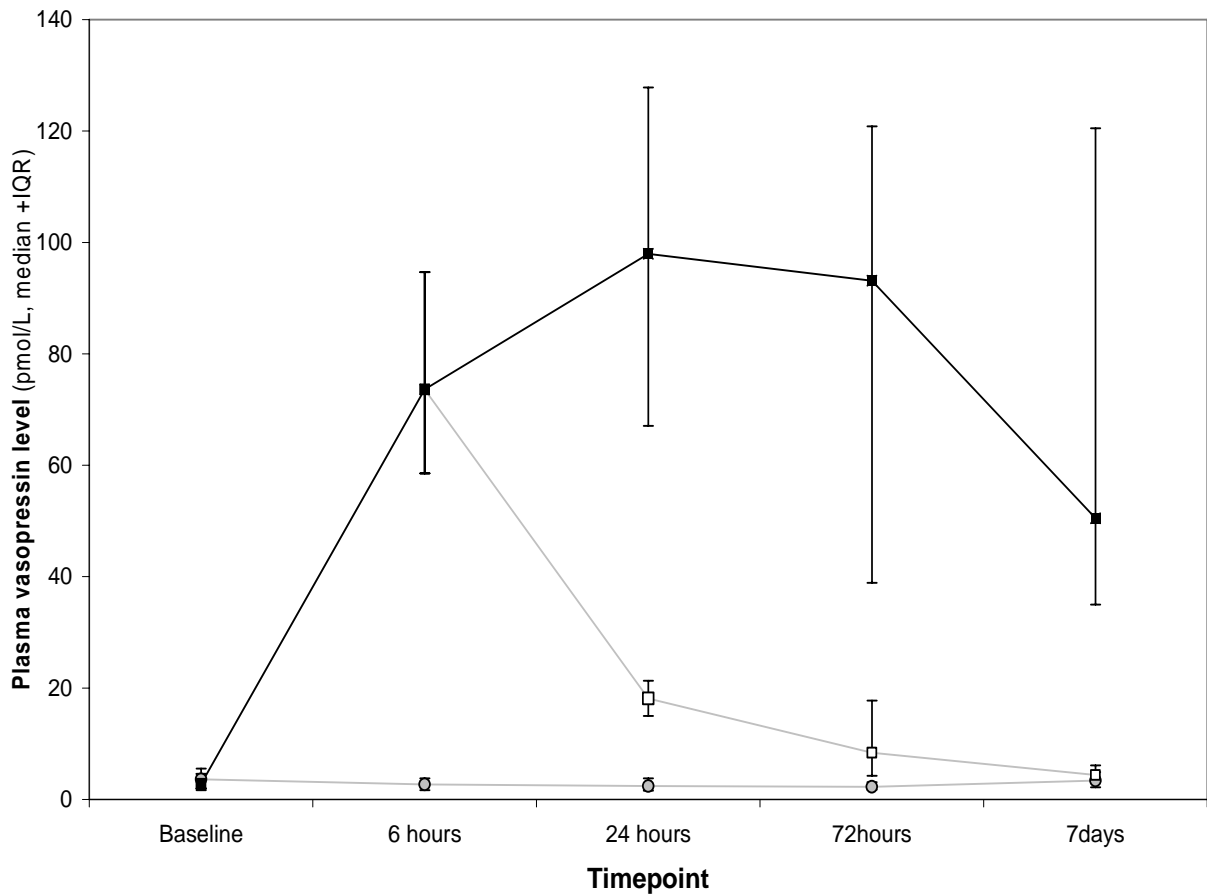


Online Figure 1. Comparison of mean arterial pressure (above) and heart rate (below) in the norepinephrine group (grey circles) and vasopressin group (black squares). Values are mean +/- standard deviation. Heart rate was significantly lower in the vasopressin group than in the

norepinephrine group over the first four days ($P < 0.001$). There were no statistically significant differences between the norepinephrine and vasopressin groups in mean arterial pressure.



Online Figure 2. Rates of total norepinephrine infusion (open-label and study drug) in the vasopressin treated group (black squares) and the norepinephrine treated group (grey circles) amongst patients who were treated only with open-label norepinephrine at baseline. The rates of norepinephrine infusion were significantly lower in the vasopressin group than in the norepinephrine group over the first four days ($P < 0.001$). Values are median + interquartile range



Online Figure 3. Plasma vasopressin levels over time in a convenience sample of patients receiving a vasopressin infusion, n = 54, (black squares), patients in the vasopressin group once vasopressin infusions had stopped (open squares) and patients in the norepinephrine group, n = 53, (grey circles). Values are median + interquartile range. For reference, physiologic plasma vasopressin levels are typically less than 4 pmol/L in humans who are not hypotensive (Cowley AW, Cushman WC, Quillen EW, et al. Vasopressin elevation in essential hypertension and increased responsiveness to sodium intake. *Hypertension* 1981; 3:I93-I100).

ONLINE TABLE 2. DEMOGRAPHICS AND BASELINE CHARACTERISTICS OF PATIENTS ACCORDING TO SEVERITY OF SEPTIC SHOCK

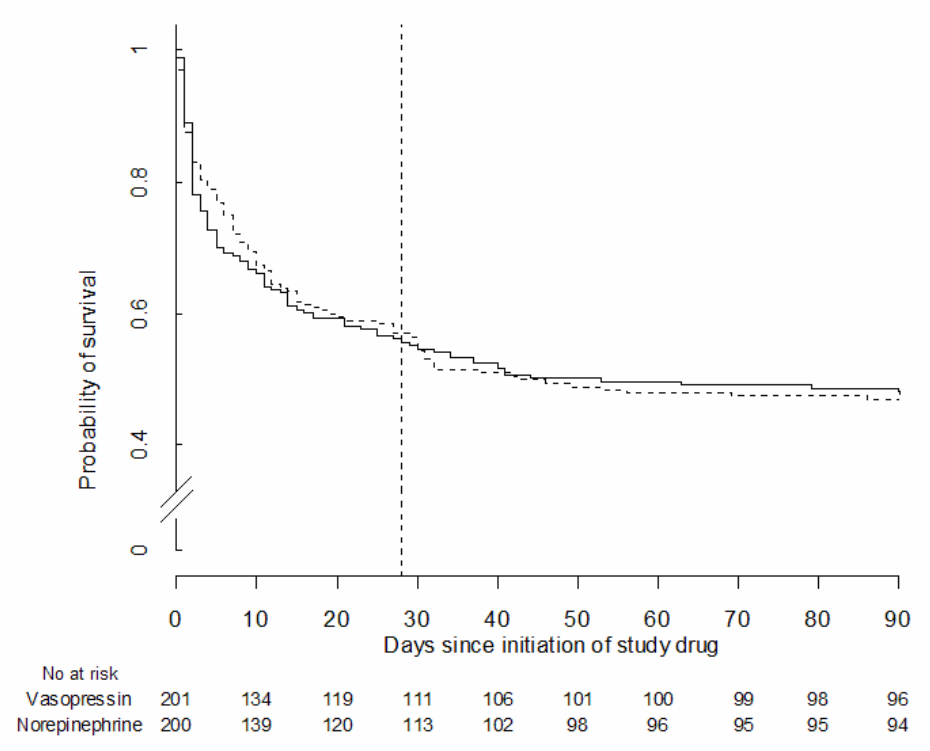
| CHARACTERISTIC | LESS SEVERE SEPTIC SHOCK N (%) N = 378 | MORE SEVERE SEPTIC SHOCK N (%) N= 401 | P VALUE |
|------------------------------------|---|--|---------|
| Age (years) | 60.5 ± 16.2 | 60.6 ± 16.3 | 0.99 |
| Male Gender | 229 (60.6) | 246 (61.3) | 0.83 |
| Recent surgical history | 141 (37.3) | 142 (35.4) | 0.59 |
| APACHE II score | 25.3 ± 7.2 | 28.7 ± 7.1 | <0.001 |
| Ethnicity | | | |
| Caucasian | 324 (85.7) | 332 (82.8) | 0.36 |
| Pre-existing conditions | | | |
| Ischemic Heart Disease | 65 (17.2) | 68 (17.0) | 0.62 |
| Congestive Heart Failure | 36 (9.5) | 22 (5.5) | 0.30 |
| COPD | 74 (19.6) | 53 (13.2) | 0.04 |
| Chronic Renal Failure | 40 (10.6) | 48 (12.0) | 0.51 |
| Diabetes | 73 (19.3) | 92 (22.9) | 0.28 |
| Liver Disease | 43 (11.4) | 45 (11.2) | 0.62 |
| Alcoholism | 61 (16.1) | 47 (11.7) | 0.13 |
| Injection Drug Abuse | 22 (5.8) | 12 (3.0) | 0.10 |
| Cancer | 89 (23.5) | 100 (24.9) | 0.56 |
| Immunocompromised | 61 (16.1) | 78 (19.5) | 0.30 |
| Solid Organ Transplant | 15 (4.0) | 16 (4.0) | 0.62 |
| Steroid use | 86 (22.8) | 82 (20.4) | 0.47 |
| Recent Trauma | 18 (4.8) | 21 (5.2) | 0.76 |
| New Organ Failure | | | |
| Cardiovascular | 378 (100) | 401 (100) | 1.0 |
| Respiratory | 331 (87.6) | 352 (87.8) | 0.85 |
| Renal | 231 (61.1) | 291 (72.6) | 0.001 |
| Hematology/Coagulation | 85 (22.5) | 117 (29.2) | 0.03 |
| Neurologic | 82 (21.7) | 108 (26.9) | 0.15 |
| Number of organ dysfunctions | 3.3 ± 1.1 | 3.6 ± 1.1 | 0.001 |
| Source of Infection | | | 0.03 |
| Lung | 177 (46.8) | 150 (37.4) | |
| Abdomen | 92 (24.3) | 119 (29.7) | |
| Other* | 109 (28.8) | 132 (32.9) | |
| Pathogen Type in positive cultures | | | 0.54 |
| Gram positive alone | 64 (16.9) | 75 (18.7) | |
| Gram negative alone | 34 (9.0) | 49 (12.2) | |
| Mixed organisms | 144 (38.1) | 138 (34.4) | |
| Other & unknown | 136 (36.0) | 139 (34.7) | |
| Hemodynamic variables | | | |

| | | | |
|---|----------------------|------------------------|--------|
| Systolic Blood Pressure (mm Hg) | 111.6 ± 16.2 | 107.2 ± 17.0 | <0.001 |
| Mean Arterial Pressure (mm Hg) | 74.0 ± 9.2 | 71.6 ± 9.7 | 0.03 |
| Arterial pH | 7.35 ± 0.08 | 7.29 ± 0.11 | 0.11 |
| Serum lactate (mmol/L) | 2.4 ± 2.0 | 4.3 ± 3.6 | <0.001 |
| Vasoactive Drug Dosage at Randomization | | | |
| Norepinephrine (µg/min) | 9.8 ± 5.5 (n=310) | 30.0 ± 23.3 (n=363) | <0.001 |
| Epinephrine (µg/min) | 7.8 ± 6.1 (n=8) | 12.6 ± 15.1 (n=42) | 0.14 |
| Dopamine (µg/kg/min) | 7.3 ± 6.0 (n=19) | 7.5 ± 5.8 (n=35) | 0.92 |
| Dobutamine (µg/kg/min) | 4.7 ± 3.1 (n=24) | 6.2 ± 5.0 (n=65) | 0.09 |
| Milrinone (µg/kg/min) | 0.3 ± 0.1 (n=17) | 0.4 ± 0.3 (n=16) | 0.36 |
| Phenylephrine (µg/min) | 110 ± 71 (n=68) | 188 ± 76 (n=88) | <0.001 |
| Time from meeting inclusion criteria to study drug infusion (hours) | 12.3 ± 10.3 | 11.2 ± 7.9 | 0.08 |

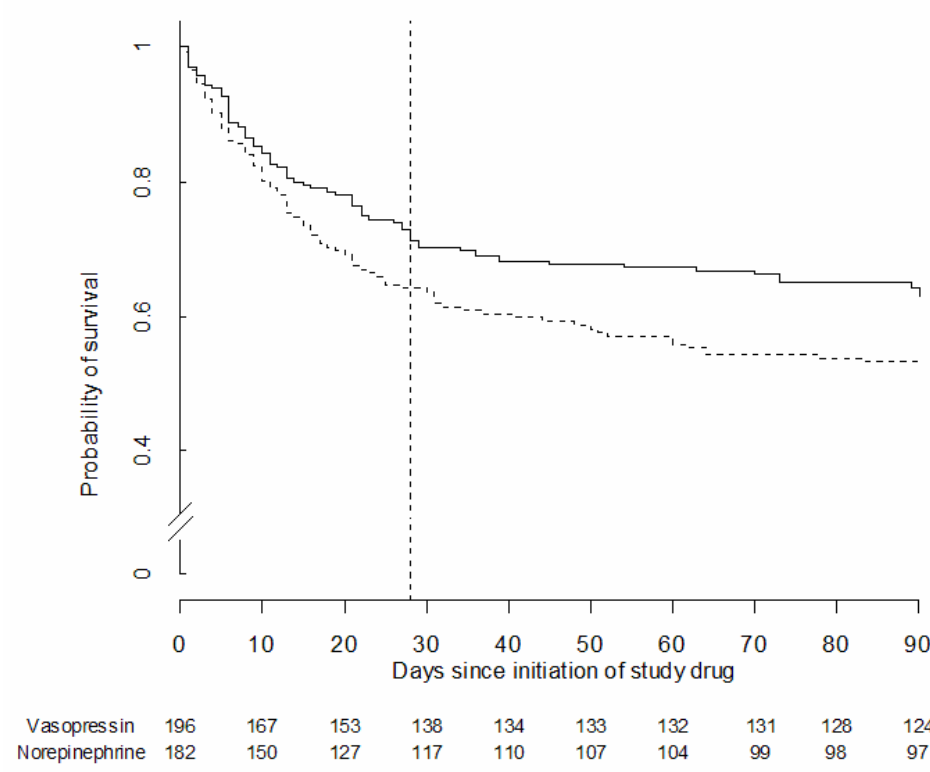
Plus-minus values are means ± SD. COPD denotes chronic obstructive pulmonary disease, and

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*Other sites of infection included the blood, skin, central nervous system, bones and joints, cardiac system and reproductive organs



A.



B.

Online Figure 4. 90-day Kaplan Meier Survival Curves, A) patients in more severe stratum, ($p = 0.77$ at day 28 and $p = 0.92$ at day 90), B) patients in less severe stratum ($p = 0.05$ at day 28 and $p = 0.03$ at day 90). The interaction p value (test for heterogeneity) was 0.10. Solid black line is the vasopressin treated group, the dotted line is the norepinephrine treated group, and the vertical line marks day 28. P values were calculated using the log rank statistic.

ONLINE TABLE 3. ANALYSIS OF THE RATES AND RISKS OF DEATH FROM ANY CAUSE IN PATIENTS HAVING SEPTIC SHOCK ACCORDING TO ADDITIONAL POST-HOC SUBGROUPS

| Post-hoc subgroups | | NOREPINEPHRINE GROUP N / total N (%) | VASOPRESSIN GROUP N / total N (%) | P VALUE* | INTERACTION STATISTIC P VALUE** |
|---|---|--|---|-----------------|--|
| Treatment by lactate Quartile | First lactate quartile (≤ 1.4 mmol/L) | 26/77 (33.8) | 17/90 (18.9) | 0.03 | 0.04 |
| | Second lactate quartile (1.5 – 2.3 mmol/L) | 36/87 (41.4) | 35/85 (41.2) | 0.98 | 0.65 |
| | Third lactate quartile (2.4 – 4.4 mmol/L) | 30/77 (39.0) | 31/85 (36.5) | 0.74 | 0.51 |
| | Fourth lactate quartile (> 4.4 mmol/L) | 47/83 (56.6) | 49/80 (61.3) | 0.55 | - |
| Treatment by number of vasopressors at baseline | One vasopressor | 108/271 (39.9) | 85/272 (31.3) | 0.04 | 0.04 |
| | Two or more vasopressors | 42/111 (37.8) | 55/124 (44.4) | 0.31 | - |
| Treatment by use of norepinephrine at baseline | Norepinephrine | 123/329 (37.4) | 120/343 (35.0) | 0.52 | 0.31 |
| | No norepinephrine | 27/53 (50.9) | 20/53 (37.7) | 0.17 | - |
| Treatment by pathogen identification | Pathogen identified | 112/292 (38.4) | 117/324 (36.1) | 0.56 | 0.35 |
| | No Pathogen identified | 38/90 (42.2) | 23/72 (31.9) | 0.18 | - |
| Treatment by APACHE II Quartiles | APACHE II < 22 | 23/95 (24.2) | 24/116 (20.7) | 0.54 | 0.73 |
| | APACHE II 23-27 | 39/119 (32.8) | 32/92 (34.8) | 0.76 | 0.59 |
| | APACHE II 28-32 | 34/81 (42.0) | 34/100 (34.0) | 0.27 | 0.57 |

| | | | | | |
|--|----------------|------------------|------------------|------|------|
| | APACHE II >32 | 54/87 (62.1) | 50/88 (56.8) | 0.48 | - |
| Treatment by APACHE II ≤ 27 versus >27 | APACHE II ≤ 27 | 62/214 (29.0) | 56/208 (26.9) | 0.64 | 0.50 |
| | APACHE II >27 | 88/168 (52.4) | 84/188 (44.7) | 0.15 | - |
| Treatment by Time to Study Drug Infusion | ≤ 12 hrs | 90/222 (40.5) | 68/205 (33.2) | 0.12 | 0.28 |
| | >12 hrs | 60/160 (37.5) | 72/191 (37.7) | 0.97 | - |

* Within subgroup analysis, two-sided P values are based on Pearson's chi-squared tests.

**Interaction statistic was calculated using logistic regression.