

Supplemental online content

A multinational randomized trial of mega-dose esomeprazole as anti-inflammatory agent in sepsis

Table of contents

<i>Members of PPI-SEPSIS Study Group</i>	2
<i>PPI-SEPSIS Study Committees</i>	5
<i>Definitions</i>	6
<i>Handling of missing data</i>	8
<i>Post hoc logistic analysis</i>	9
<i>In vitro studies</i>	10
<i>Table S1. Sequential Organ Failure Assessment (SOFA) score</i>	12
<i>Table S2. Simplified Acute Physiology Score (SAPS) II</i>	13
<i>Table S3. Prohibited concomitant treatments</i>	15
<i>Table S4. PPI-SEPSIS inclusion and exclusion criteria</i>	16
<i>Table S5. Ethics Committee approval for each participating center</i>	17
<i>Figure S1. Monthly patient enrolment during the study period</i>	19
<i>Figure S2. Sequential Organ Failure Assessment score during the first 10 days after randomization</i>	20
<i>Figure S3. Kaplan-Meier analysis for mortality at day 90</i>	21
<i>Figure S4. Risk reduction for the primary outcome associated with study treatment in subgroups, expressed as mean difference (95% confidence interval)</i>	22
<i>Table S5. Univariate analyses of the association of baseline variables with the outcome of 90-day mortality</i>	24
<i>Table S6. Multiple logistic regression analysis for 90-day mortality</i>	25
<i>Table S7. Per protocol analysis</i>	26
<i>Table S8. Sensitivity analysis including only collected data without imputation</i>	28
<i>Figure S5. Primary outcome in each participating center</i>	30
<i>Figure S6. Secretion of inflammatory (IL1-β, TNF-α, IL-6 and HMGB1) and anti-inflammatory (IL-10 and IL1Ra) markers in monocytes from control (CTR) and patients (PZ) after LPS stimulation</i>	31
<i>Figure S7. Levels of intracellular ROS (A) and cysteine release (B) in untreated (UNT) or LPS stimulated monocytes from controls (CTR) and patients (PZ)</i>	32
<i>Figure S8. I Inhibitory effect of esomeprazole on IL-1β secretion, ROS levels and cysteine release in monocytes from sepsis patients</i>	33
<i>Figure S9. Total antioxidant capacity in the serum of patients pre- and post-with placebo or esomeprazole (ESO)</i>	34
<i>Figure S10. Levels of IL-1-β (A), intracellular ROS (B) and cysteine release (C) in monocytes from patients pre- and post-treated with placebo or esomeprazole (ESO)</i>	35
<i>Supplemental References</i>	36

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Statistical analysis: Rosalba Lembo, M.Sc. was the biostatistician dedicated to data cleaning and final blind analyses. Cristina Nakhnoukh helped with data cleaning.

Definitions

Sepsis

Sepsis diagnosis was defined by confirmed or suspected infection and an acute change in total Sequential Organ Failure Assessment (SOFA) score ≥ 2 points (Table S1, Supplement) from baseline or from zero in the absence of a known baseline value before infection, as per Sepsis-3 definitions

Septic shock

Septic shock was defined as sepsis with persisting hypotension requiring vasopressors to maintain mean arterial pressure (MAP) ≥ 65 mmHg and a serum lactate level >2 mmol L⁻¹ (18 mg dL⁻¹) despite adequate hemodynamic resuscitation, as per Sepsis-3 definitions.

Mean daily SOFA score over 10 days

The primary endpoint of our study is organ dysfunction measured by the mean daily Sequential Organ Failure Assessment (SOFA) score over 10 days.

The SOFA score is a clinical tool used to assess and quantify the severity of organ dysfunction in critically ill patients. It evaluates dysfunction in six organ systems (respiratory, cardiovascular, hepatic, coagulation, renal, and neurological) and assigns a score based on the degree of dysfunction observed from 0 to 4 for each organ system.

The total SOFA score is derived by summing the scores from each organ system, ranging from 0 to 24. A higher total score suggests a more critical overall condition with multiple organ dysfunctions. The Mean Daily SOFA Score is determined by summing the daily SOFA scores over a specified period and dividing this total by the number of days in the considered timeframe. This calculation provides an average representation of organ dysfunction throughout the designated period, offering insights into the overall trend and severity of multi-organ impairment over time. This approach enables a more comprehensive understanding of the patient's condition by considering variations in organ dysfunction across consecutive days. If patients will die or will be discharged within the first post-randomization 10 days, no data imputation will be performed after the event of death or discharge.

28-day mortality

We defined 28-day mortality as death by any cause within the period from randomization up to 28 days.

60-day mortality

We defined 60-day mortality as death by any cause within the period from randomization up to 60 days.

90-day mortality

We defined 90-day mortality as death by any cause within the period from randomization up to 90 days.

Days alive and free from antibiotics at day 28

We defined days alive and free from antibiotics at day 28 as the number of days without any antibiotic therapy during the initial 28 days after randomization. Patients discharged before day 28 after randomization were censored at hospital discharge, and deaths within the initial 28 days were assigned zero days alive and free from antibiotics at day 28.¹⁰

Days alive and free from ICU on day 28

We defined days alive and free from intensive care unit (ICU) at day 28 as the number of days of not being in the ICU during the initial 28 days after randomization. Deaths within the initial 28 days were assigned zero days alive and free from ICU at day 28.

Length of ICU stay

Defined as time from ICU admission to ICU discharge.

Length of hospital stay

Defined as the duration of hospital stay from hospital admission to hospital discharge.

Handling of missing data

The calculation of the mean daily SOFA score requires a SOFA score for each of the ten days. Given that the mean daily SOFA score is a daily value calculated over a period of ten days, the possibility of missing data was considered. If necessary, missing data was managed as follows: when only one or two consecutive days were missing, or if the missing data occurred in one or more of the last few days, we used the last available SOFA score value. In case three or more days were missing, the average value of the last available and next available observation was used. We also conducted a sensitivity analysis including only collected data to further assess the robustness of our findings.

Post hoc logistic analysis

A post-hoc logistic regression model with stepwise selection was used to identify predictors of the survival as a sensitivity analysis with the aim of identifying residual baseline imbalances that could have masked the effect of the intervention itself. Clinical data collected before randomization were entered into the model if they had a univariate $P < 0.10$. We then repeated the analysis using a less conservative entry criterion ($P = 0.20$). Treatment allocation was forced into the multivariate model. Collinearity and overfitting were assessed with the use of a stepwise regression model and a Pearson correlation test. In the multivariate analyses, variables are expressed as odds ratios with 95% CIs.

***In vitro* studies**

Trial design

In monocytes from septic patients, we investigated inflammatory status and redox stress at a basal level and following stimulation with TLR agonist. We analyzed the effects *in vitro* of PPI on these parameters and the cellular modifications in monocytes from surviving patients treated with esomeprazole or placebo. Additionally, in the serum of these patients, we evaluated the total antioxidant capacity and their response to PPI.

Methods

Monocytes were isolated from heparinized peripheral blood obtained from 26 patients at baseline and after 72h of placebo or esomeprazole treatment and from healthy individuals using Ficoll gradient and adherence to culture plates.(1) These cells were then cultured in RPMI-1640 with 5% FCS (Euroclone) at 37°C, 5% CO₂ and stimulated for 18h with lipopolysaccharide (LPS, 100ng/ml, Sigma-Aldrich), a Toll-like receptor (TLR) agonist, alone or in combination with esomeprazole (200 µM).(2)

The supernatant from the cell cultures stimulated 18h with LPS was used to quantify specific proteins of interest using ELISA kits (R&D Systems and IBL International).(3) H₂DCF-DA staining (5 µM for 30 min at 37°C, Invitrogen) was employed to assess intracellular reactive oxygen species (ROS) production in monocytes unstimulated or treated 1h with LPS.(4) The ROS levels were expressed as relative fluorescence unit (RFU). Cysteine release was measured in the supernatant from the cell cultures untreated or stimulated for 18h with LPS using a colorimetric assay.(5) Serum was obtained from whole peripheral blood in patients at baseline and after 72h of placebo or esomeprazole treatment, and the total antioxidant capacity was evaluated following the manufacturer's instructions of a dedicated kit (MAK187, Sigma, St. Louis, MO, USA) that provides a complete description of the total cell antioxidant power associated with endogenous scavengers, expressed as Trolox equivalent antioxidant capacity content.(6) Trolox, a water-soluble vitamin E analogue, was used as an antioxidant standard.

Statistical analyses were performed using GraphPad Prism 9 (GraphPad, San Diego, CA, USA). Data are presented as mean ± SEM. Differences among the experimental conditions were tested using t test. Statistical significance was considered for p values < 0.05.

Outcomes

For the *in vitro* studies, the following outcomes were planned: a. Identification of cytokine production in septic patients' monocytes; b. Correlation between redox stress and altered cytokine production; c. Reduction of redox stress and cytokine production in septic patients' monocytes treated *in vitro* with esomeprazole; d. Differences in monocytes isolated from patients treated with placebo or esomeprazole.

Results

In activated monocytes collected from 26 patients at baseline, there was a dysregulated and increased secretion of pro-inflammatory cytokines like IL-1β, TNF-α, IL-6, and higher production of HMGB-1, an inflammatory mediator. The elevated inflammatory markers were associated with reduced levels of anti-inflammatory cytokines like IL-1Ra and IL-10 (Supplemental Figure S6).

Additionally, monocytes from these patients exhibited an altered redox state compared to healthy controls. The stress state of unstimulated sepsis monocytes is characterized by the increased ROS levels and by the higher release of free cysteine, a marker of the xc⁻ system, a major antioxidant system in monocytes (Supplemental Figure S7). Stress at baseline can result in an altered response of sepsis monocytes to LPS stimulation with unbalanced cytokine secretion. Subsequently, patients' monocytes were treated *in vitro* with esomeprazole, revealing that esomeprazole inhibited the secretion of IL-1β (n=15), ROS production (n=10), and cysteine release (n=10) (Supplemental

Figure S8). Esomeprazole reducing ROS production and antioxidant response could restore a condition of redox homeostasis that ensures correct cytokine production.

To study the effect of esomeprazole administration to patients, blood was collected at randomization and after placebo (n=10) or PPI (n=12) treatment for 72h. In such samples, we found a reduction in total antioxidant capacity (TAC) in the serum of patients treated with esomeprazole, confirming the drug's inhibitory effects on antioxidant mechanisms observed in *in vitro* monocytes (Supplemental Figure S9). The inhibitory effect of esomeprazole on TAC levels could reflect the lower production of oxidative stress, TAC levels were decreased to maintain the balance between oxidant and antioxidant state.

Further analysis involved isolating monocytes from patient blood post-treatment (72h) and examining cytokine secretion and redox status. In such studies we found no significant differences in monocytes isolated from patients treated with placebo (n=12) or esomeprazole (n=14) compared to the data obtained at baseline (Supplemental Figure S10).

Table S1. Sequential Organ Failure Assessment (SOFA) score

	0	+1	+2	+3	+4
GCS	15 points	13–14 points	10–12 points	6–9 points	< 6 points
MAP <i>or</i> vasoactive agents required	MAP $\geq$ 70 mmHg	MAP < 70 mmHg	Dopamine $\leq$ 5 mcg/kg/min <i>or</i> dobutamine (any dose)	Dopamine > 5, epinephrine $\leq$ 0.1, or norepinephrine $\leq$ 0.1 mcg/kg/min	Dopamine > 15, epinephrine > 0.1, or norepinephrine > 0.1 mcg/kg/min
PaO ₂ /FiO ₂ , mmHg	$\geq$ 400	300-399	200-299 <i>or</i> < 199 and NOT mechanically ventilated	100-199 and mechanically ventilated	< 100 and mechanically ventilated
Platelets, $\times 10^3$ mL ⁻¹	$\geq$ 150	100-149	50-99	20-49	< 20
Bilirubin, mg dL ⁻¹	< 1.2	1.2–1.9	2.0–5.9	6.0–11.9	> 12.0
Creatinine, mg dL ⁻¹ (<i>or</i> urinary output)	< 1.2	1.2-1.9	2.0-3.4	3.5-4.9 (< 500 ml day ⁻¹)	$\geq$ 5.0 (< 200 ml day ⁻¹)

GCS = Glasgow Coma Scale, MAP = mean arterial pressure, PaO₂ = arterial oxygen partial pressure, FiO₂ = fractional inspired oxygen.

Adapted from Vincent, 1996

Table S2. Simplified Acute Physiology Score (SAPS) II

Variable			Points
Age, years		<40	0
		40-59	7
		60-69	12
		70-74	15
		75-79	16
		≥80	18
Heart rate (beats minute ⁻¹)	Worst value in 24 hours; if patient has had both cardiac arrest (11 points) and extreme tachycardia (7 points), assign 11 points	<40	11
		40-69	2
		70-119	0
		120-159	4
		≥160	7
Systolic BP, mmHg	Worst value in 24 hours	<70	13
		70-99	5
		100-199	0
		≥200	2
Temperature ≥39°C (102.2°F)	Highest temperature in 24 hours	No	0
		Yes	3
GCS	Lowest value in 24 hours; if patient is sedated, use estimated GCS before sedation	14-15	0
		11-13	5
		9-10	7
		6-8	13
		<6	26
PaO ₂ /FiO ₂ , if on mechanical ventilation or CPAP	Lowest value in 24 hours; if patient was extubated <24 hours ago, use lowest value while on mechanical ventilation	<100	11
		100-199	9
		≥200	6
		Not on mechanical ventilation or CPAP within the last 24 hours	0
BUN, mg dL ⁻¹ or serum urea, mmol L ⁻¹	Highest value in 24 hours	BUN <28 Urea <10	0
		BUN 28-83 Urea 10-29.6	6
		BUN ≥84 or urea ≥30	10
Urine output, mL day ⁻¹	If patient in ICU <24 hours, calculate for 24 hours (e.g. if 1 L in 8 hours, then mark 3 L in 24 hours)	<500	11
		500-999	4
		≥1,000	0
Sodium, mEq L ⁻¹ or mmol L ⁻¹	Worst value in 24 hours	<125	5
		125-144	0
		≥145	1
Potassium, mEq L ⁻¹	Worst value in 24 hours	<3.0	3
		3.0-4.9	0
		≥5.0	3
Bicarbonate, mEq L ⁻¹	Lowest value in 24 hours	<15	6
		15-19	3
		≥20	0

Bilirubin, mg dL ⁻¹ (μmol L ⁻¹)	Highest value in 24 hours	<4.0 (<68.4)	0
		4.0-5.9 (68.4-102.5)	4
		≥6.0 (≥102.6)	9
WBC, x 10 ³ /mm ³	Worst value in 24 hour	<1.0	12
		1.0-19.9	0
		≥20.0	3
Chronic disease		None	0
		Metastatic cancer	9
		Hematologic malignancy	10
		AIDS	17
Type of admission	Scheduled surgical = surgery scheduled ≥24 hours in advance Medical = no surgery within one week of admission Unscheduled surgical = surgery scheduled ≤24 hours in advance	Scheduled surgical	0
		Medical	6
		Unscheduled surgical	8

BP = blood pressure, GCS = Glasgow Coma Scale, CPAP = continuous positive airway pressure, BUN = blood urea nitrogen, ICU = intensive care unit, WBC = white blood cells.
Adapted from Le Gall, 1993.

Table S3. Prohibited concomitant treatments.

Pharmacologic class	Active principle
Antiviral agents	Atazanavir Atazanavir / cobicistat Dolutegravir / rilpivirine Emtricitabine / nelfinavir / tenofovir Nelfinavir Rilpivirine Sofosbuvir / velpatasvir Sofosbuvir / velpatasvir / voxilaprevir
Tyrosine kinase inhibitors	Acalabrutinib Dasatinib Erlotinib Neratinib Pazopanib
Others	Citalopram Clopidogrel Methotrexate Tacrolimus

Table S4. PPI-SEPSIS inclusion and exclusion criteria.

Inclusion criteria (All the following must be present)	Exclusion criteria (Patient is excluded if ≥ 1 of the following is present)
1. Age ≥ 18 years old 2. Admitted to ICU or ED 3. Sepsis ^a or septic shock ^b 4. Able to express informed consent or as requested by Ethical Committee	1. Able to express informed consent and deny it 2. Known allergy or intolerance to study drug 3. Little chance of survival, with SAPS II >65 points 4. Concomitant HIV infection with a CD4 count <200 cells/ μ L 5. On immunosuppressant or long-term corticosteroid therapy (more than 0.5 mg/kg/day of prednisone or equivalent for over 30 days) 6. Receiving lifesaving drugs known to have a strong interference with esomeprazole as stated in <i>Table 2: Prohibited concomitant treatments</i> 7. Sepsis or septic shock since over 36 hours 8. Severe hepatic dysfunction 9. Ongoing pregnancy

ICU = intensive care unit, ED = emergency department, SAPS = Simplified Acute Physiology Score, HIV = human immunodeficiency virus.

^aDefined as acute change in total Sequential Organ Failure Assessment (SOFA) score ≥ 2 points consequent to the infection. The baseline SOFA score can be assumed to be zero in patients not known to have preexisting organ dysfunction.

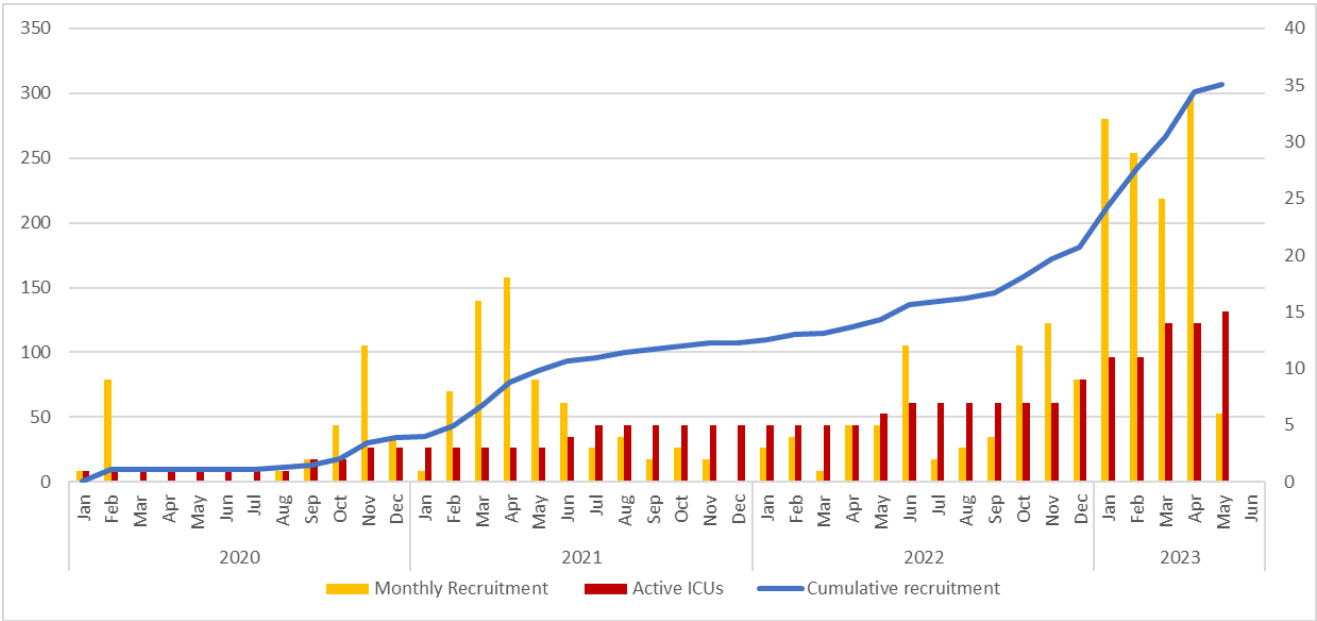
^bDefined as sepsis plus persisting hypotension requiring vasopressors to maintain mean arterial pressure ≥ 65 mmHg and having a serum lactate level >2 mmol/L (18 mg/dL) despite adequate volume resuscitation.

Table S5. Ethics Committee approval for each participating center

Country	City	Site	Ethics Committee name	Approval number	Approval date
Italy	Milan	IRCCS San Raffaele Hospital	Comitato Etico IRCSS Ospedale San Raffaele	XXI	05/04/2018
Italy	Catanzaro	A.O.U. Mater Domini	Comitato Etico Regione Calabria Sezione Area Centro	29/INT/2018	23/04/2018
Italy	Pisa	Azienda Ospedaliero Universitaria Pisana	Comitato Etico Regione Toscana – Area Vasta Nord Ovest CE150034	16163_CORRADI	12/09/2019
Italy	Rome	Università Campus Bio-Medico di Roma	Comitato Etico dell’Università Campus Bio-Medico di Roma	08.20	19/02/2020
Italy	Pisa	Azienda Ospedaliero Universitaria Pisana	Comitato Etico Regione Toscana – Area Vasta Nord Ovest CE150034	16163_GUARRACINO	08/10/2020
Italy	Udine	Azienda Sanitaria Universitaria Friuli Centrale	Comitato Etico Unico Regionale	CEUR-2020-SPER-112	01/02/2021
Italy	Rozzano	Humanitas Research Hospital	Comitato Etico Indipendente Istituto Clinico Humanitas	702/21	30/09/2021
Italy	Turin	A.U.O. Città della Salute e della Scienza di Torino	Comitato Etico Interaziendale AOU Città della Salute e della Scienza di Torino	362/2021	08/11/2021
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Russian Federation	Moscow	Federal Research and Clinical Center of Intensive Care Medicine and	Ethics Committee of Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology	2/22/2	26/07/2022

		Rehabilitology			
Russian Federation	Moscow	V. A. Negovskiy Reanimatology Research Institute	Ethics Committee of Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology	2/22/2	26/07/2022
Italy	San Donà di Piave	Ospedale San Donà di Piave – ULSS 4 Veneto Orientale	Comitato Etico per la Sperimentazione Clinica della Provincia di Venezia e IRCCS San Camillo (CESC)	233A/CESC	30/08/2022
Italy	Merano	Ospedale di Merano	Comitato Etico dell’Azienda Sanitaria della Provincia Autonoma di Bolzano	92-2022	21/09/2022
Kazakhstan	Astana	Astana Medical University	Local Commission on Bioethics RSE on REM “NNTsTO im. Academician N.D. Batpenov” MH RK	3	27/09/2022
Italy	Lodi	ASST Lodi – Ospedale Maggiore di Lodi	Comitato Etico Milano Area 1	2022/ST/162	07/02/2023
Italy	Potenza	AOR San Carlo	Comitato Etico Unico Regionale per la Basilicata	20230019006	24/04/2023

Figure S1. Monthly patient enrolment during the study period.



The right-hand scale presents the number of patients enrolled per month; the left-hand scale presents the total number of patients enrolled.

Figure S2. Sequential Organ Failure Assessment score during the first 10 days after randomization

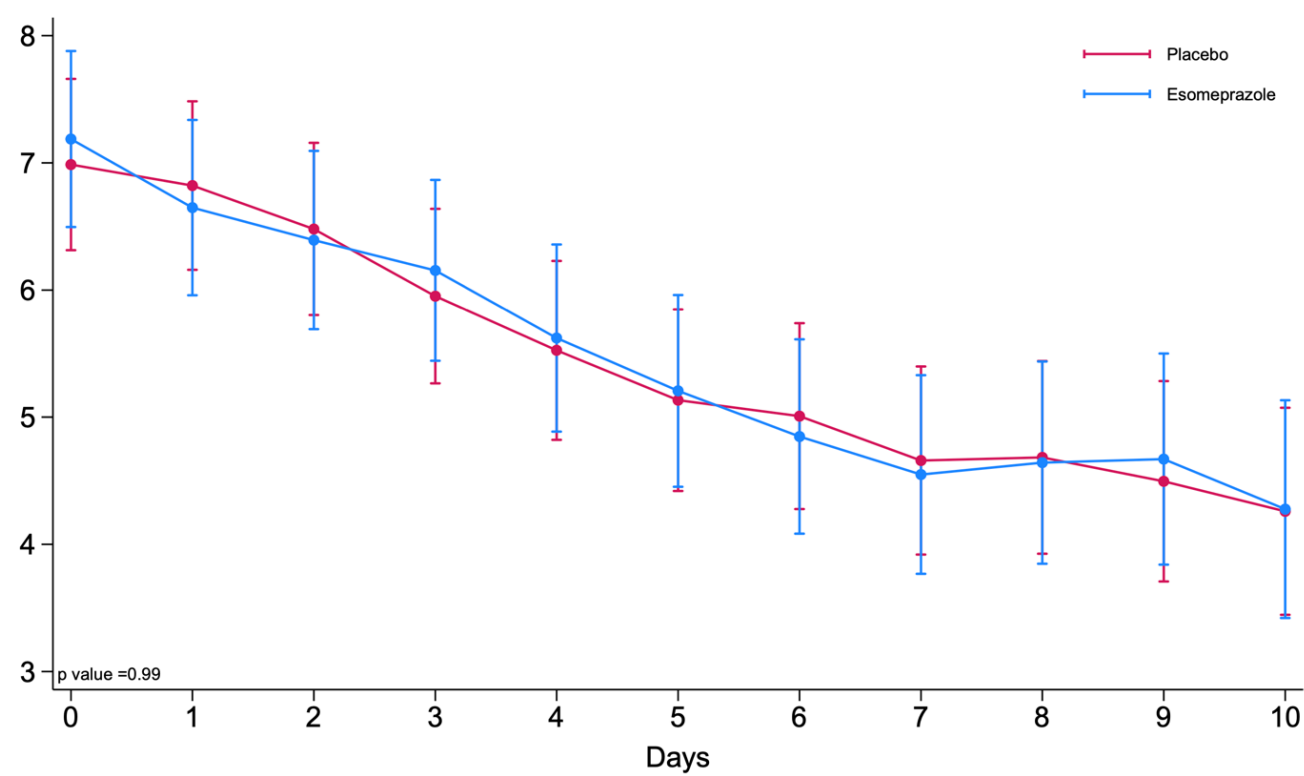


Figure S3. Kaplan-Meier analysis for mortality at day 90

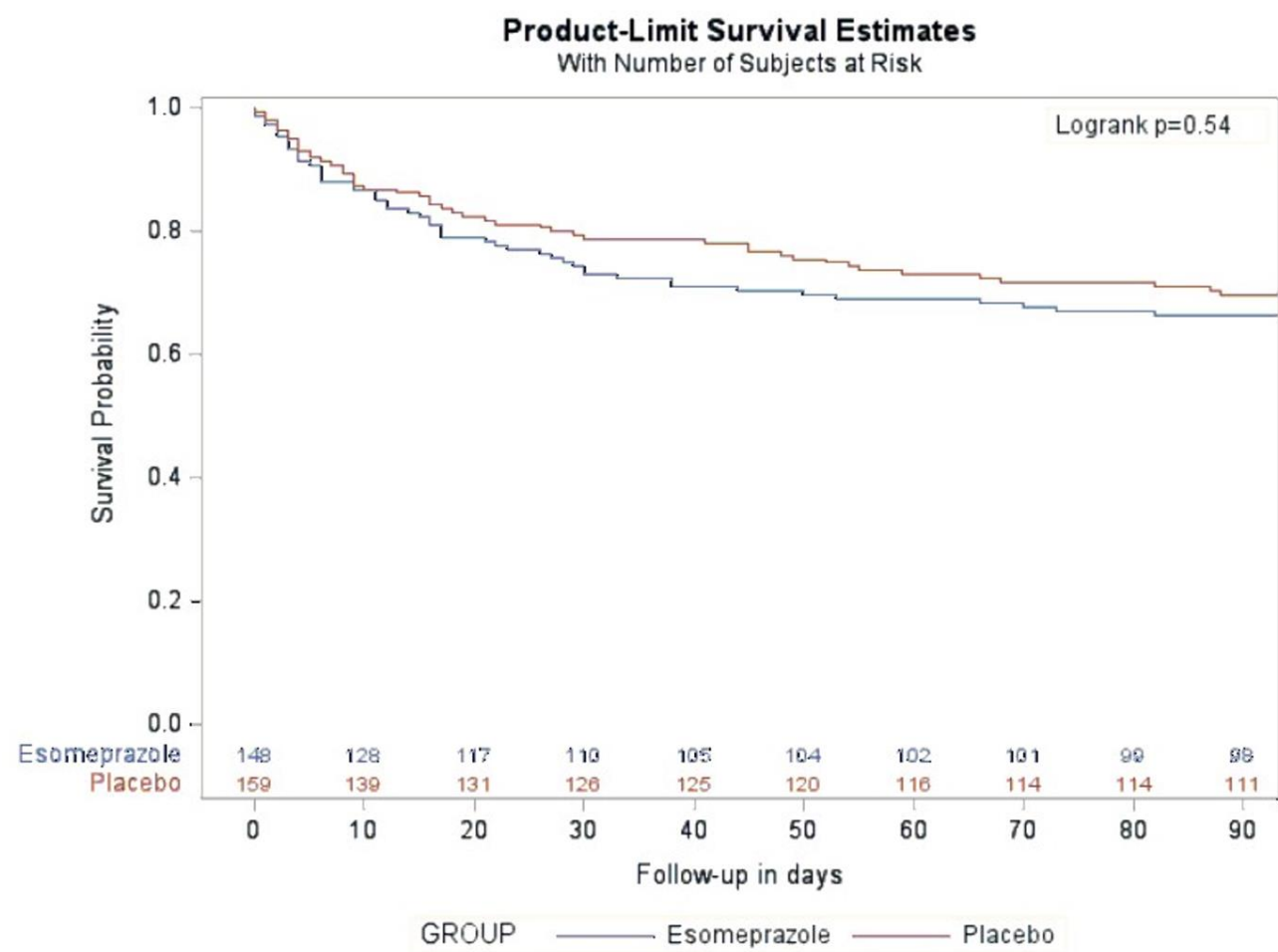
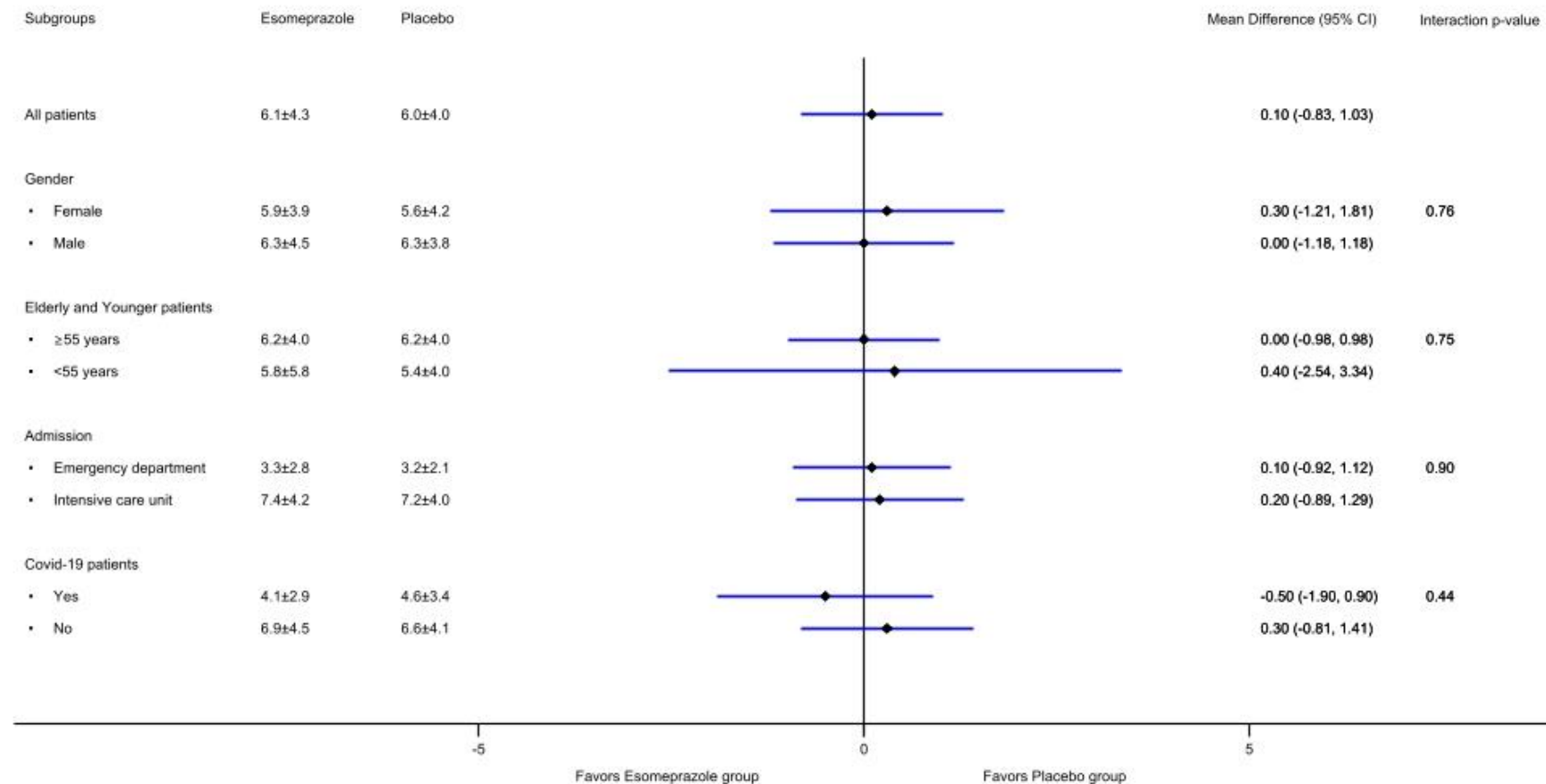
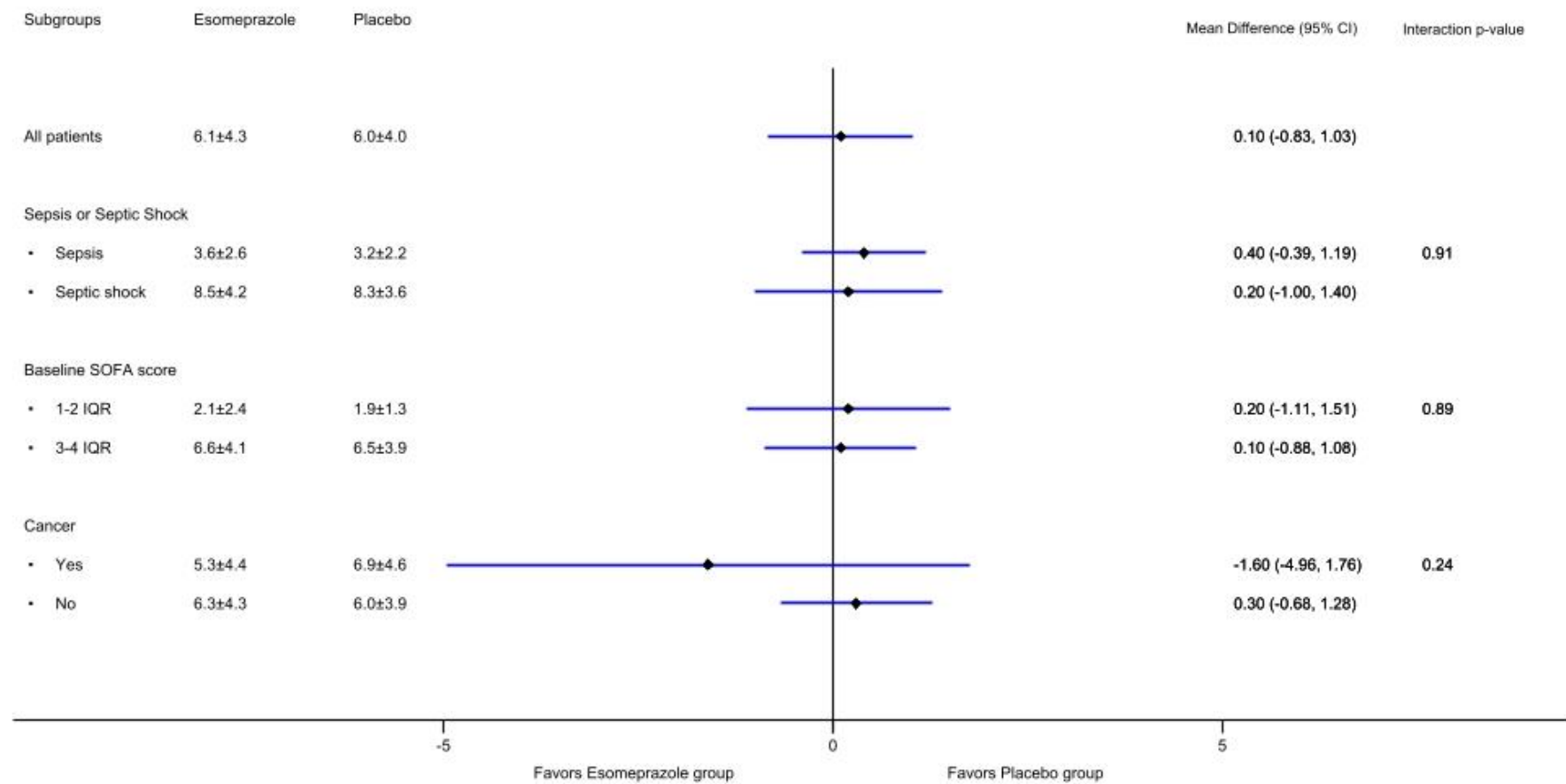


Figure S4. Risk reduction for the primary outcome associated with study treatment in subgroups, expressed as mean difference (95% confidence interval)





SOFA = Sequential Organ Failure Assessment, CI = confidence interval, IQR = interquartile range.

Table S5. Univariate analyses of the association of baseline variables with the outcome of 90-day mortality.

	Overall (n=307)	Deceased at 90 days (n=100)	Alive at 90 days (n=207)	P
Median age (IQR), y	71 (61-78)	74 (65-80)	69 (59-76)	0.02
Male sex, n (%)	196 (63.8)	66 (66)	130 (62.8)	0.61
Median body mass index ^a (IQR), kg/m ²	26.3 (23.9 - 29.8)	25.2 (22.8 - 28.7)	27.4 (24.4 - 31.0)	<0.01
Year of randomization				0.47
2020	34 (11.1)	11 (11.0)	23 (11.1)	
2021	73 (23.8)	18 (18.0)	55 (26.6)	
2022	74 (24.1)	28 (28.0)	46 (22.2)	
2023	126 (41)	43 (43.0)	83 (40.1)	
Race or ethnic group, n (%)				0.18
White	296 (96.4)	97 (97.0)	199 (96.1)	
Asian	5 (1.6)	0 (0.0)	5 (2.4)	
Hispanic	5 (1.6)	2 (2.0)	3 (1.5)	
African American	1 (0.3)	1 (1.0)	0 (0.0)	
Comorbidities ^b , n (%)				
Hypertension	195 (63.9)	65 (65.0)	130 (63.4)	0.80
Smoking history	78 (25.7)	26 (26.0)	52 (25.5)	1.00
Dyslipidemia	110 (36.1)	48 (48.0)	62 (30.2)	<0.01
Diabetes	85 (27.9)	27 (27.0)	58 (28.3)	0.89
CAD	49 (16.1)	24 (24.0)	25 (12.2)	0.01
COPD	42 (13.8)	19 (19.2)	23 (11.2)	0.08
Cerebrovascular disease	31 (10.2)	15 (15.0)	16 (7.8)	0.07
Active cancer	30 (9.8)	14 (14.0)	16 (7.8)	0.10
Congestive heart failure	30 (9.8)	15 (15.0)	15 (7.3)	0.04
Hepatic impairment	18 (5.9)	12 (12.1)	6 (2.9)	<0.01
Hematologic malignancy	7 (2.3)	4 (4.0)	3 (1.5)	0.16
Asthma	6 (2)	0 (0.0)	6 (2.9)	0.09
Hepatic cirrhosis	2 (0.7)	2 (2.0)	0 (0.0)	0.04
Clinical presentation, n (%)				<0.01
Sepsis	141 (45.9)	27 (27.0)	114 (55.1)	
Septic shock	166 (54.1)	73 (73.0)	93 (44.9)	
Median SOFA score	7 (4 - 10)	9 (5 - 12)	5 (3 - 9)	<0.01
Randomized in the ICU, n (%)	217 (70.9)	77 (77.8)	140 (67.6)	0.08

IQR = interquartile range, CAD = coronary artery disease, COPD = chronic obstructive pulmonary disease, SOFA = Sequential Organ Failure Assessment, ICU = intensive care unit.

^aCalculated as weight in kilograms divided by height in meters squared

^bThe proportion of missing values was less than 5%

Table S6. Multiple logistic regression analysis for 90-day mortality.

Variable	OR (CI)	P
Dyslipidemia	1.72 (1.02-2.97)	0.04
SOFA score at baseline	1.15 (1.07-1.23)	<0.01
Hepatic impairment	4.3 (1.35-13.71)	0.01
Body mass index ^a	0.94 (0.90-0.99)	0.01
Group of randomization	1.48 (0.87-2.53)	0.14

Entry significance criterion of $p < 0.2$

OR = Odds ratio, CI = confidence interval, SOFA = Sequential Organ Failure Assessment.

^aCalculated as weight in kilograms divided by height in meters squared.

Table S7. Per protocol analysis

	Overall (n=292)	Mega-dose esomeprazole group (n=140)	Placebo group (n=152)	Absolute risk difference (95% CI)	<i>P</i>
Primary outcome					
Median of mean SOFA score over 10 days ^a (IQR)	5 (3-9)	4 (3-9)	5 (3-8)	0 (-0.9; 0.9)	0.81
Secondary outcomes					
Median of single organ failure severity ^{a,b} (IQR)					
Central nervous system	0 (0-2)	0 (0-2)	0 (0-2)	-0.1 (-0.4; 0.2)	0.62
Cardiovascular	3 (0-4)	3 (0-4)	3 (0-4)	0.1 (-0.3; 0.5)	0.52
Respiratory	2 (1-2)	2 (1-2)	2 (1-2)	0 (-0.2; 0.2)	0.97
Coagulation	0 (0-1)	0 (0-1)	0 (0-1)	0 (-0.2; 0.2)	0.76
Liver	0 (0-0)	0 (0-0)	0 (0-0)	0 (-0.1; 0.2)	0.96
Renal function	0 (0-1)	0 (0-2)	1 (0-1)	0.2 (-0.1; 0.4)	0.54
Follow up at day 28					
Median antibiotic-free days, median (IQR), <i>d</i>	14 (0-21)	15 (0-21)	14 (0-21)	0.9 (-1.5; 3.2)	0.51
Median ICU-free days ^c (IQR), <i>d</i>	10 (0-22)	13 (0-22)	9 (0-22)	0.6 (-2.2; 3.3)	0.80
Median length of ICU stay ^c (IQR), <i>d</i>	11 (5-23)	10 (5-21)	12 (5-25)	-3.4 (-8.3; 1.5)	0.29
Median length of hospital stay ^a (IQR), <i>d</i>	17 (10-33)	16 (9-28)	18 (11-37)	-2.7 (-7.9; 2.4)	0.19
Median LOS in ICU among survivors ^c (IQR), <i>d</i>	10 (5-20)	9 (5-16)	12 (5-24)	-2.1 (-7.9; 3.8)	0.29
Median LOS in hospital among survivors ^a (IQR), <i>d</i>	17 (11-33)	16 (10-27)	18 (12-37)	-2.8 (-8.5; 3.0)	0.19
Readmission to ICU ^a , <i>n</i> (%)	20 (7.1)	9 (6.6)	11 (7.6)	-1.0 (-7.0; 5.0)	0.75
Mortality, <i>n</i> (%)					
At 28 days	54 (18.5)	29 (20.7)	25 (16.4)	4.3 (4.7; 13.2)	0.35
At 60 days	73 (25.0)	38 (27.1)	35 (23.0)	4.1 (5.8; 14.1)	0.42
At 90 days	86 (29.5)	44 (31.4)	42 (27.6)	3.8 (6.1; 14.3)	0.48

Data are presented as risks difference for dichotomous outcomes and as absolute mean differences for continuous outcomes. The risk difference is calculated as the event probability in the Mega-dose esomeprazole group minus the event probability in the Placebo group. The absolute mean difference is calculated as the mean of the Mega-dose esomeprazole group minus the mean of the Placebo Group. The 95% confidence intervals presented in this table have not been adjusted for multiplicity; therefore, inferences drawn from these intervals may not be reproducible.

SOFA = Sequential Organ Failure Assessment, IQR = interquartile range, ICU = intensive care unit, LOS = length of stay.

^aThe proportion of missing values was less than 5%

^bMeasured by single mean daily SOFA score variables over 10 days

^cData missing from 33 and 31 subjects in the treatment and placebo group, respectively;

Table S8. Sensitivity analysis including only collected data without imputation.

	Overall (n=307)	Mega-dose esomeprazole group (n=148)	Placebo group (n=159)	Absolute Risk difference (95% CI)	P value
Primary outcome					
Median of mean SOFA score over 10 days ^a (IQR)	5 (3-9)	5 (3-9)	5 (3-9)	0.1 (-0.8; 1.0)	0.99
Secondary outcomes					
Median of single organ failure severity ^{a,b} (IQR)					
Central nervous system	0 (0-2)	0 (0-2)	0 (0-2)	0 (-0.3; 0.3)	0.87
Cardiovascular	3 (0-4)	3 (0-4)	3 (0-4)	0.1 (-0.3; 0.5)	0.39
Respiratory	2 (1-2)	2 (1-3)	2 (1-3)	0 (-0.2; 0.2)	1.00
Coagulation	0 (0-1)	0 (0-1)	0 (0-1)	0.1 (-0.1; 0.2)	0.76
Liver	0 (0-0)	0 (0-1)	0 (0-0)	0 (-0.1; 0.2)	0.95
Renal function	0 (0-1)	0 (0-2)	1 (0-1)	0.1 (-0.1; 0.4)	0.53
Follow up at day 28					
Median antibiotic-free days (IQR), <i>d</i>	14 (0-21)	15 (0-21)	13 (0-21)	0.7 (-1.6; 3.0)	0.62
Median ICU-free days ^c (IQR), <i>d</i>	8 (0-22)	9 (4-19)	8 (0-21)	0.4 (-2.3; 3.0)	0.91
Median length of ICU stay ^c (IQR), <i>d</i>	10 (4-21)	9 (4-19)	12 (4-24)	-3.4 (-8.1; 1.3)	0.26
Median length of hospital stay ^a (IQR), <i>d</i>	16 (10-33)	15 (9-27)	17 (11-36)	-2.8 (-7.8; 2.2)	0.18
Median LOS in ICU among survivors ^c (IQR), <i>d</i>	10 (5-20)	9 (5-16)	12 (5-24)	-2.1 (-7.9; 3.8)	0.29
Median LOS in hospital among survivors ^a (IQR), <i>d</i>	17 (11-33)	16 (10-27)	18 (12-37)	-2.8 (8.5; 3.0)	0.15
Readmission to ICU ^a , <i>n</i> (%)	20 (6.8)	9 (6.3)	11 (7.2)	-1.0 (-6.7; 4.7)	0.74
Early death within 24 hours, <i>n</i> (%)	8 (2.6)	5 (3.4)	3 (1.9)	1.5 (-2.1; 5.1)	0.49
Mortality, <i>n</i> (%)					
At 28 days	69 (22.5)	37 (25.0)	32 (20.1)	4.9 (-4.5; 14.2)	0.31
At 60 days	88 (28.7)	46 (31.1)	42 (26.4)	4.7 (-5.5; 14.8)	0.37
At 90 days	101 (32.9)	52 (35.1)	49 (30.8)	4.3 (-6.2; 14.8)	0.42

Data are presented as risks difference for dichotomous outcomes and as absolute mean differences for continuous outcomes. The risk difference is calculated as the event probability in the Mega-dose esomeprazole group minus the event probability in the Placebo group. The absolute mean difference is calculated as the mean of the Mega-dose esomeprazole group minus the mean of the Placebo Group. The 95% confidence intervals presented in this table have not been adjusted for multiplicity; therefore, inferences drawn from these intervals may not be reproducible.

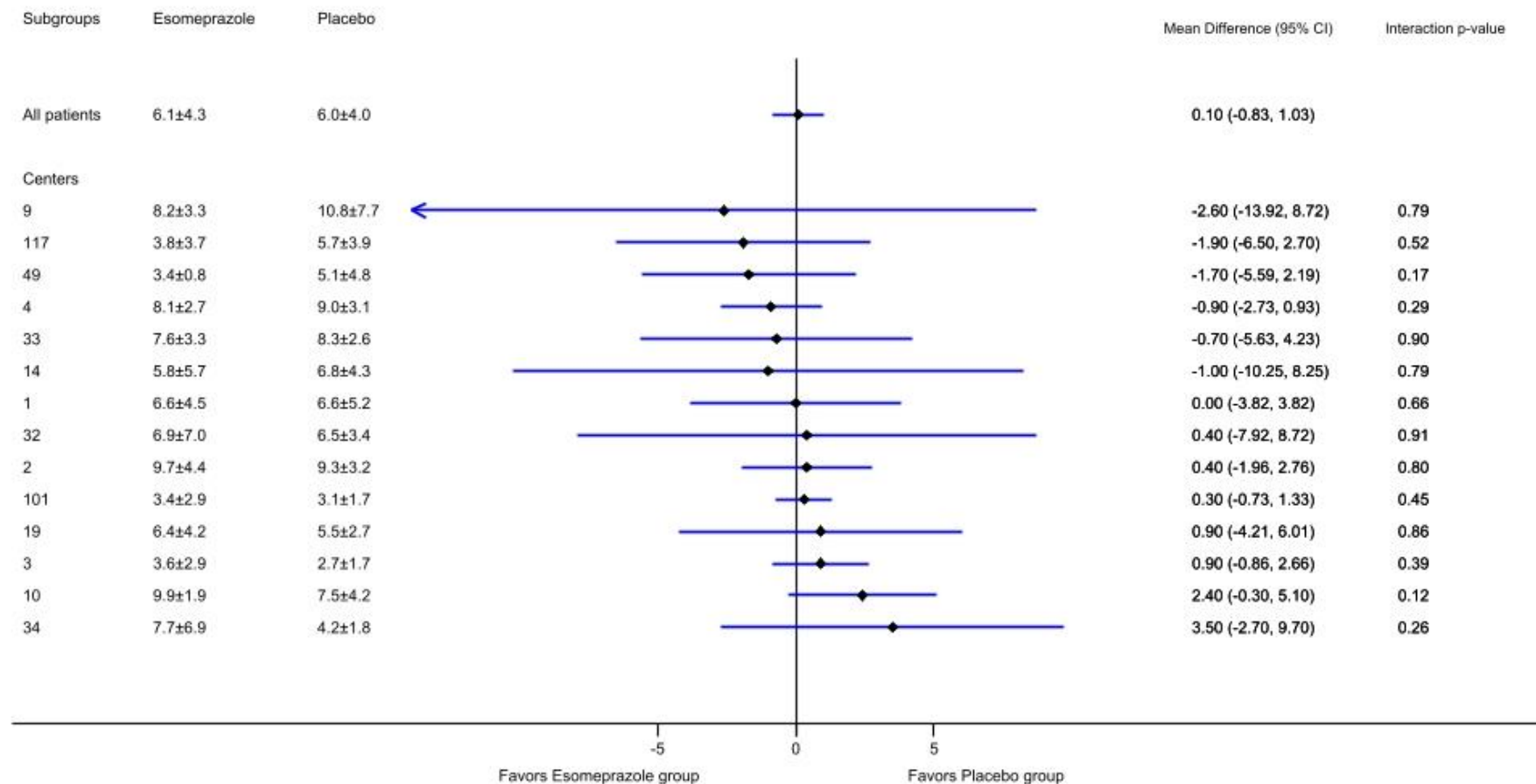
SOFA = Sequential Organ Failure Assessment, IQR = interquartile range, ICU = intensive care unit, LOS = length of stay.

^aThe proportion of missing values was less than 5%

^bMeasured by single mean daily SOFA score variables over 10 days

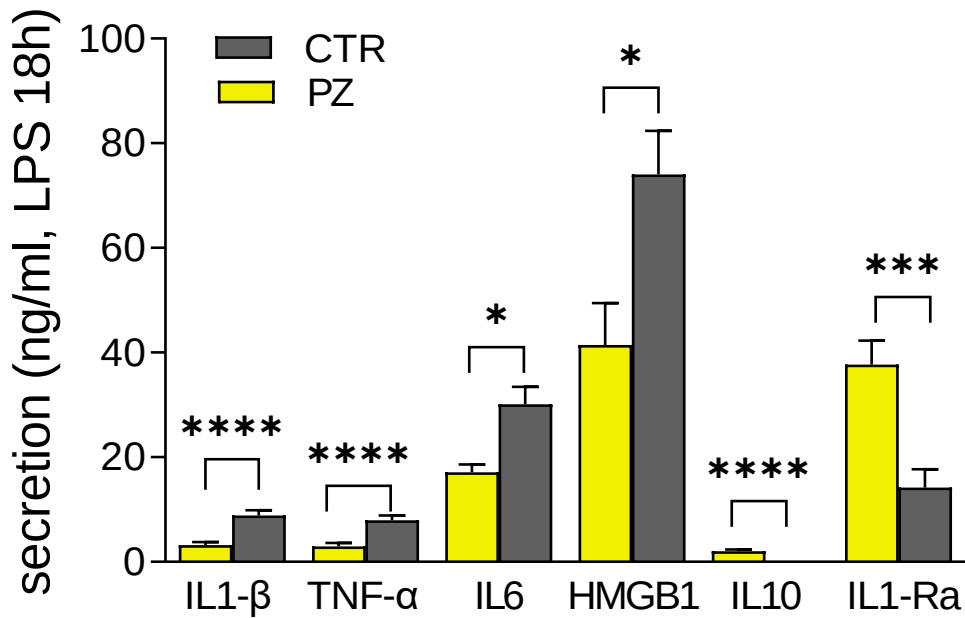
^cData missing from 33 and 31 subjects in the treatment and placebo group, respectively

Figure S5. Primary outcome in each participating center



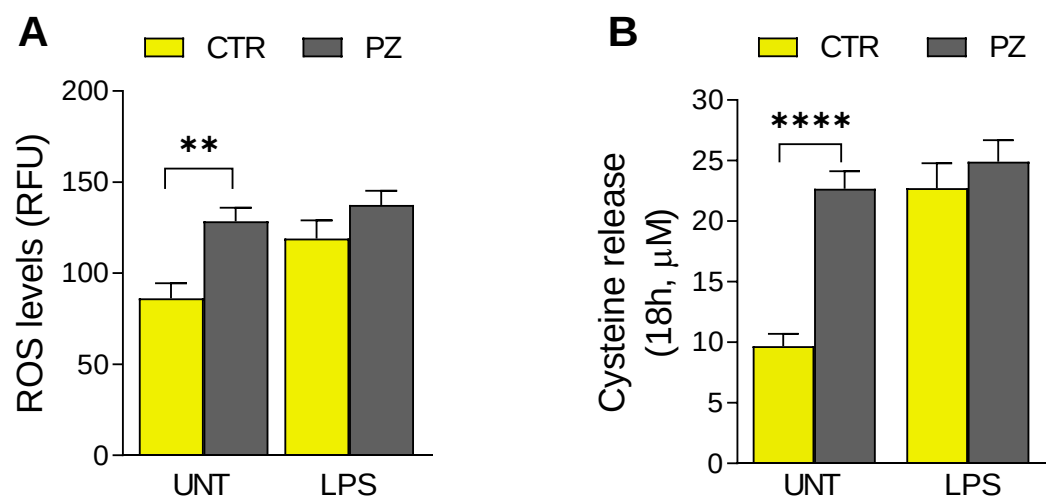
CI = confidence interval.

Figure S6. Secretion of inflammatory (IL1- β , TNF- α , IL-6 and HMGB1) and anti-inflammatory (IL-10 and IL1Ra) markers in monocytes from control (CTR) and patients (PZ) after LPS stimulation.



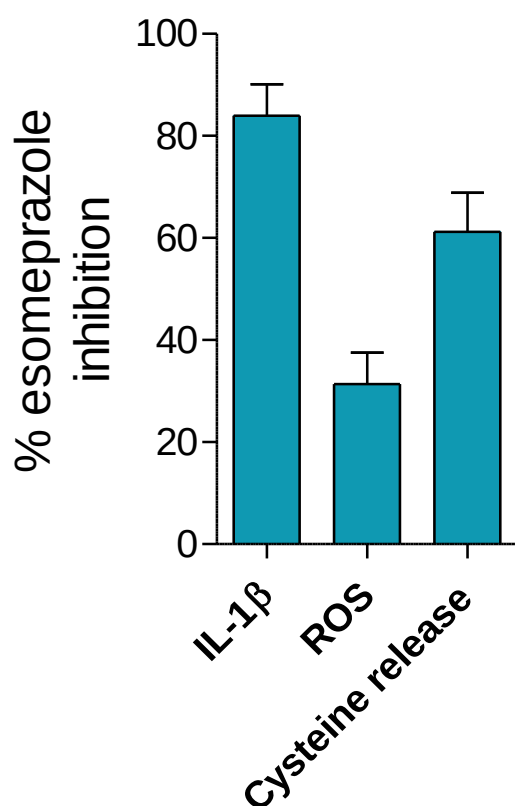
* $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$

Figure S7. Levels of intracellular ROS (A) and cysteine release (B) in untreated (UNT) or LPS stimulated monocytes from controls (CTR) and patients (PZ).



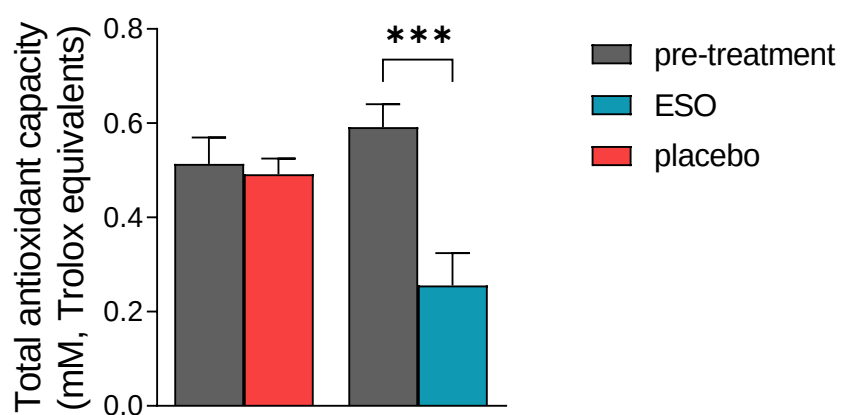
** p < 0.01, **** p < 0.0001

Figure S8. I Inhibitory effect of esomeprazole on IL-1 β secretion, ROS levels and cysteine release in monocytes from sepsis patients.



Data are expressed as percentual of inhibition, mean $\pm$ SEM.

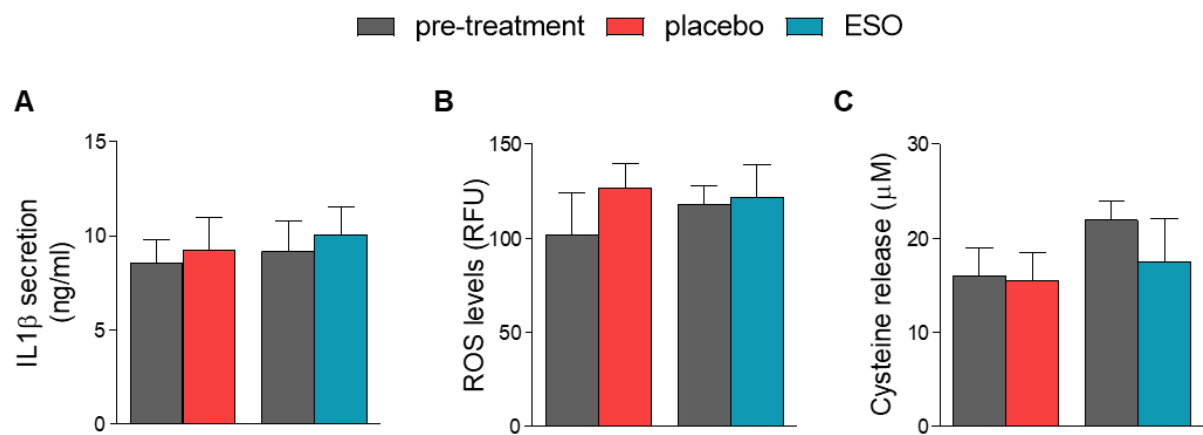
Figure S9. Total antioxidant capacity in the serum of patients pre- and post-with placebo or esomeprazole (ESO).



*** $p < 0.001$.

Trolox, a water-soluble vitamin E analogue, was used as an antioxidant standard.

Figure S10. Levels of IL-1- β (A), intracellular ROS (B) and cysteine release (C) in monocytes from patients pre- and post-treated with placebo or esomeprazole (ESO).



Supplemental References

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