

The Effects of Atorvastatin on Inflammatory Responses and Mortality in Septic Shock: A Single-center, Randomized Controlled Trial

Ratender Kumar Singh, Vikas Agarwal¹, Arvind Kumar Baronia, Sudeep Kumar², Banani Poddar, Afzal Azim

Departments of Critical Care Medicine, ¹Immunology and ²Cardiology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India

Abstract

Aim of the Study: Pleiotropic effect of statins can modulate inflammation in septic shock. We tested the hypothesis whether statins can reduce mortality in septic shock. **Patients and Methods:** We conducted a randomized double-blinded trial with treatment (40 mg dose of atorvastatin for 7 days) and control (placebo) arm in adult septic shock patients admitted to the Intensive Care Unit. Primary (28-day mortality) and secondary (vasopressor-, ventilation-, and renal replacement therapy-free days) outcomes, with lipid profile and adverse effects, were documented. Inflammatory biomarkers (interleukin [IL]-1, IL-6, tumor-necrosis-factor [TNF]- α , interferon [IFN], and C-reactive protein [CRP]), were also measured before (day 1 [D1]) and after start of trial drug (D4 and D7). **Results:** Seventy-three septic shock patients with 36 and 37 included in the atorvastatin and placebo group, respectively. Both groups were equally matched. Twenty-eight-day mortality, event-free days, lipid profile, and adverse effects were also not significantly different between groups. Reduced levels of IL-1, IL-6, TNF- α , IFN, and CRP were observed in the atorvastatin group. Also observed were significant day-wise changes in inflammatory biomarkers. **Conclusions:** Atorvastatin-induced changes in inflammatory biomarkers did not confer mortality benefit in septic shock (ClinicalTrials.gov/NCT02681653).

Keywords: Inflammatory biomarkers, mortality, septic shock, statins

INTRODUCTION

Sepsis is life-threatening organ dysfunction caused by a dysregulated host response to infection.^[1] Septic shock is a subset of sepsis, in which underlying circulatory and cellular/metabolic abnormalities are profound enough to substantially increase mortality.^[1] In spite of advancement in management of septic shock in the last two decades, mortality remains unacceptably high. Furthermore, escalating cost of intensive care with limited health insurance in developing countries is only aggravating the problem. These conditions continue to fuel search for new adjunctive therapies in septic shock to reduce mortality, cost of therapy, and length of stay.

Statins (hydroxymethyl glutaryl coenzyme A reductase inhibitors) have been shown to have anti-inflammatory and immunomodulatory properties capable of attenuating inflammatory response in sepsis.^[2-4] Their lipid-lowering capabilities have been well acknowledged specifically in

patients at risk of atherosclerotic cardiovascular events. Pleiotropic properties of statins in the prevention and mitigation of sepsis have also been intensely investigated. In a meta-analysis of 26 observational studies on infection and sepsis, statins were associated with a significant decrease in mortality.^[5] However, in stark contrast to these observational studies, the meta-analysis of randomized controlled trials (RCTs) on this issue did not report any survival benefit.^[5,6]

Despite superiority over observational studies, existing RCTs are also limited by profound variability. The variability exists

Address for correspondence: Dr. Ratender Kumar Singh, Department of Critical Care Medicine, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Raebareli Road, Lucknow - 226 014, Uttar Pradesh, India.
E-mail: ratender@sgpgi.ac.in

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in dose, duration, and type of statin, primary and secondary outcomes, severity of sepsis, prior statin use, and nonuniform quantification of adverse effects of statins. Furthermore, impact of statins in an exclusive cohort of septic shock has never been previously studied. To test the hypothesis that statins have survival benefit in septic shock, we conducted a prospective randomized double-blinded phase II trial with treatment (atorvastatin) and control (placebo) arm. The present study has used the older definitions of sepsis, severe sepsis, and septic shock as were prevalent at the time of conception, design, implementation, and completion of the study.^[7-9]

PATIENTS AND METHODS

The study was conducted in a tertiary care referral hospital and academic institute in north of India. A combined adult pediatric medical and surgical Intensive Care Unit (ICU) of 12 beds with an annual admission rate of 250 was used for this purpose.

Ethics, consent, and permissions

The study was approved and funded by the ethics (A-02:PGI/IMP/IEC/56/19.8.11) and research committee of the institute, respectively. Written informed consent was obtained from patients or their surrogates as appropriate.

Study design

The study was a prospective, randomized, double-blinded placebo-controlled trial to assess the impact of atorvastatin in septic shock patients. The trial was registered with the ClinicalTrials.gov with registration number NCT02681653.

Study population

Screening, enrollment, and randomization of the study population were as illustrated in Figure 1.

Inclusion criteria

Adult patients of 18 years and above in septic shock and admitted to the ICU were included in the study. Septic shock

was defined according to the Surviving Sepsis Campaign Guidelines of 2012.^[7]

Exclusion criteria

Patients were excluded from the study on grounds of prior statin use in previous 3 months of inclusion in the study, myopathy or rhabdomyolysis, severe liver disease (acute liver failure, chronic liver disease [Child-Pugh Classification C]), serum alanine aminotransferase (ALT) level >2.5 times the upper limit of normal (ULN) or a raised serum creatine kinase (CK) level higher than the ULN, chronic renal failure, anticipated gut intolerance (e.g., postoperative abdominal surgery), pregnant or breastfeeding females, concurrent drug administration (cimetidine, warfarin, ketoconazole, macrolides, immunosuppressants, except steroids, HIV protease inhibitors, and other lipid-lowering drugs), or refusal for consent.

Randomization

Patients were randomized to receive either atorvastatin or placebo (methylcellulose). Randomization was performed in 1:1 ratio using a computer-generated random number list (conducted by a person not involved in the study). Trial packs of drug were prepared by an independent pharmacy and contained atorvastatin or an equally matched placebo in size, shape, color, and weight. Allocation concealment was by sealed opaque envelopes. Participants, caregivers, investigators, and outcome assessors were all blinded to the study group assignment.

Study protocol and intervention

The patients were included in the study within 24 h of having met the inclusion criteria. Trial drug was administered through nasogastric tube after randomization and was continued daily for 7 days of ICU admission, discharge, or death whichever occurred earlier. Patients were treated with either 40 mg of atorvastatin or placebo. Patients received the first trial drug dose at the earliest opportunity (but not exceeding 24 h) of randomization. The trial drug was dissolved in 20 ml of distilled water before being given enterally.

Data collection

All patients' data were codified and stored using a study identification number on a single computer in a protected database. Basic demographic data included age, gender, coexisting medical conditions, concurrent medications, and severity scores (acute physiology and chronic health evaluation II [APACHE-II] score and sequential organ failure assessment [SOFA] score). Life support measures and other ICU interventions, vasopressors (type, dose, and duration), invasive ventilation (ventilation support, PaO₂/FiO₂ ratio, and PaCO₂), and renal replacement therapy (RRT) (modality ± diuretics [dose]) were recorded. Physiological parameters of hemodynamics (heart rate and mean arterial pressure [mmHg]), central venous oxygen saturation [%] and lactate [mg/dL]), acid-base status, urinary output, and fluid balance were also recorded. Baseline

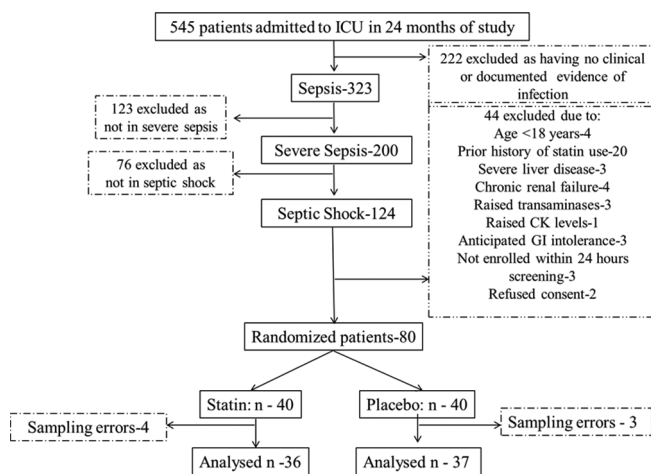


Figure 1: Flow diagram of randomization of study population. CK: Creatine kinase; GI: Gastrointestinal

biochemical measurements including lipid profile, CK, liver function tests, complete blood count, renal function tests (serum creatinine [Scr], blood urea nitrogen [BUN]), arterial blood gas (pH, PaO₂, PaCO₂, Na⁺, K⁺, Cl⁻, and HCO₃⁻), procalcitonin, and cultures were recorded. Blood samples were collected before (day 1 [D1]) and after (D4 and D7) starting of trial drug for the measurement of inflammatory biomarkers interleukin (IL)-1, 6, tumor-necrosis-factor alpha (TNF- α), interferon (IFN) and C-reactive protein (CRP). Lipid profiles (nonfasting, total cholesterol [TC], triglycerides, high-density lipoprotein, low-density lipoprotein [LDL] and very LDL) were measured on D1, D4, and D7 by enzymatic colorimetric method. BD Biosciences manufactured kits with catalog numbers for IL-1 (557953), IL-6 (555220), TNF (555212), IFN (555142), and CRP by nephelometry technique on the Siemens BN ProSpec machine were used for estimation.

Drug discontinuation

Each patient was assessed on the day of randomization, before (D1) and after starting of trial drug (D4 and D7) and then subsequently on D14, D21, and D28 if they survived in the ICU. Trial drug was discontinued if drug-induced intolerance, hypersensitivity reaction, hepatitis, or rhabdomyolysis occurred. The definition in our study for suspected drug-induced intolerance such as hepatitis and rhabdomyolysis was as follows: Drug-induced hepatitis was considered if there was rise in serum ALT level to more than twice the initial value and drug-induced rhabdomyolysis was considered if after starting the trial drug, serum CK levels increased equal to 10 times the normal value. Trial drug was also discontinued in the case of gastrointestinal intolerance as suggested by a gastric residual volume >50% of enteral intake per feed or >500 ml in previous 24 h despite use of prokinetic medications.

Septic shock management

Septic shock management was similar in the two groups, except for the use of trial drug. Fluids, vasopressors, inotropes, steroids, blood transfusions, antimicrobials and source control for sepsis, mechanical ventilation, RRT, sedation, nutrition and glycemic control, and thromboprophylaxis were in accordance with the Surviving Sepsis Campaign Guidelines of 2012.^[7] Detailed description of this management is provided as a Supplementary File.

Study outcomes

The primary endpoint of this study was mortality at 28 days of study inclusion. Secondary outcomes included length of ICU stay, event-free (vasopressor-free, ventilation-free, and renal replacement-free) 28 days, effect on inflammatory biomarkers, lipid profile, and adverse effects of the trial drug.

Sample size and statistical analysis

Sample size

Group sample size of 28 each in drug and placebo group was needed to achieve a power of 80%, with the aim to determine an absolute difference between group proportions of 0.30. The

proportion in trial drug group was assumed to be 0.50 under the null hypothesis and 0.80 under the alternative hypothesis. The proportion in placebo group was 0.50. The test statistic used was the one-sided Z-test with unpooled variance. The significance level of the test was targeted at 0.05. We assumed a follow-up loss of about 10%, so finally the sample size for each group was 31. Power analysis and sample size (NCSS [2008] PASS, Kaysville, UT) was used to estimate the sample size for this study.

Statistical analysis

Normality of continuous data was tested using Shapiro–Wilk test. Nonnormal, continuous data were expressed as median (interquartile range), while categorical data were expressed as frequency and percentage. Mann–Whitney U-test was used to compare the medians between the drug and placebo group. Chi-square test was used to compare the proportions/test the association between groups. For repeated observations over time, Friedman analysis of variance (ANOVA) was used to estimate the significance level among the time points. If, in Friedman ANOVA, the *P* value was observed to be significant, then the difference in medians between individual groups was further assessed using the Wilcoxon signed rank test. A two-tailed *P* < 0.05 was considered statistically significant. IBM, SPSS version 23 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

RESULTS

The study was conducted over 24 months from January 2012 to December 2014. Five hundred and forty-five patients were admitted to the ICU during this period. Approximately 59%, 37%, and 23% patients were in sepsis, severe sepsis, and septic shock, respectively. The flow diagram of randomization of the study population is as depicted in Figure 1. Sixteen percent (20/124) prior statin users were excluded from the study. On account of sampling errors, four included patients had to be deleted from the final analysis. These sampling errors included those samples which were inadequate in volume or improperly collected, labeled, stored, transported, or misplaced. Finally, there were 36 and 37 patients in septic shock who were randomly allocated to receive atorvastatin or placebo, respectively. As D1 represents results from baseline (i.e., no trial drug treatment), then D4 represents a maximum of three possible doses and D7 represents a maximum of six possible doses. The atorvastatin and placebo group received a median of 4 (1–7) and 3 (1–7) doses of atorvastatin and placebo group, respectively, during the trial week. All patients included received the drug and placebo successfully enterally.

Baseline patient characteristics

Both atorvastatin and placebo group were equally matched in terms of age, gender, coexisting illnesses, source of sepsis, and severity of illness (APACHE-II and SOFA) [Table 1].

Laboratory and physiological indices

The hematological, coagulation, and liver and renal function laboratory parameters are as depicted in Table 2. Hemoglobin,

Table 1: Baseline characteristics of septic shock patients

Patient characteristics	All patients (n=73)	Atorvastatin group (n=36)	Placebo group (n=37)	P
Age (years)	48 (33-56)	50 (38-59)	46 (28-55)	0.14
Gender female, n (%)	22 (30)	10 (45)	12 (55)	0.67
Coexisting illness, n (%)	41 (56)	21 (58)	20 (54)	0.55
Diabetes mellitus	12 (16)	7 (19)	5 (14)	0.49
Arterial hypertension	17 (23)	9 (25)	8 (22)	0.73
Chronic renal failure	3 (4)	2 (6)	1 (3)	0.22
Coronary artery disease	5 (7)	2 (6)	3 (8)	0.31
COPD	3 (4)	0	3 (8)	0.22
Chronic heart failure	1 (1)	1 (2.8)	0 (0)	0.31
Source of sepsis, n (%)				0.19
Respiratory	23 (32)	9 (25)	14 (38)	
Intra-abdominal	21 (29)	14 (39)	7 (19)	
Tropical illness	10 (14)	5 (14)	5 (14)	
Urogenital	6 (8)	5 (14)	1 (3)	
Neurological	5 (7)	1 (3)	4 (11)	
Hemato-oncological	1 (1)	0	1 (3)	
Unidentified source	7 (10)	2 (6)	5 (14)	
Severity scores				
APACHE-II				
D1	16 (13-21)	16 (13-21)	15 (12-21)	0.80
D4	16 (10-19)	16 (9-19)	16 (11-22)	0.86
D7	15 (8-18)	16 (9-18)	12 (8-18)	0.57
SOFA				
D1	12 (9-14)	12 (9-14)	11 (8-14)	0.87
D4	10 (7-13)	10 (5-12)	10 (8-14)	0.23
D7	7 (3-14)	7 (2-14)	8 (4-12)	0.81

All measurements are in median (IQR) unless specified. COPD: Chronic obstructive pulmonary disease; IQR: Interquartile range; APACHE: Acute Physiology and Chronic Health Evaluation; SOFA: Sequential organ failure assessment

Table 2: Day-wise comparison of laboratory indices between groups

Variables (laboratory)	Day 1		P	Day 4		P	Day 7		P
	Statin group (n=36)	Placebo group (n=37)		Statin group (n=36)	Placebo group (n=37)		Statin group (n=36)	Placebo group (n=37)	
Hemoglobin (g/dL)	8.3 (7.5-9.6)	8.8 (8-9.9)	0.23	7.8 (7.3-8.7)	8.4 (7.4-8.9)	0.53	8.3 (7.6-9.5)	8.8 (7.6-9.8)	0.45
WBC ($\times 10^3/\mu\text{L}$)	17.4 (11-22)	16.5 (11-21.7)	0.33	14.8 (10.7-20.4)	13.9 (8.9-24.1)	0.93	14.1 (10.4-18.5)	13.4 (9.5-17.2)	0.71
Platelet ($\times 10^3/\mu\text{L}$)	153 (85-262)	123 (80-212)	0.59	140 (58-195)	152 (71-222)	0.71	160 (83.8-236)	179 (119-297)	0.23
PT-test (s)	16.2 (14.4-17.6)	17.5 (15.1-20.3)	0.16	15.8 (14.3-17.2)	15.9 (15-17.7)	0.70	15.5 (13.3-17.2)	15.3 (14.3-17.1)	0.86
APTT-test (s)	33.95 (30.1-38.9)	34 (32.2-45)	0.30	34.7 (30.6-40.1)	39.6 (30.2-44.9)	0.29	33.6 (29.6-38.6)	35.5 (30.4-38.7)	0.41
Total bilirubin (mg/dL)	1.10 (0.7-2.4)	1.45 (0.9-2.4)	0.64	0.82 (0.7-1.5)	2.57 (0.7-6)	0.62	2.2 (0.8-6.9)	1.18 (0.5-1.7)	0.19
Direct bilirubin (mg/dL)	0.85 (0.3-2.0)	0.55 (0.3-1.1)	0.41	0.40 (0.2-1.3)	0.53 (0.2-4.1)	0.43	0.5 (0.4-1.2)	0.4 (0.3-0.9)	0.75
Indirect bilirubin (mg/dL)	0.50 (0.2-0.8)	0.6 (0.3-0.9)	0.61	0.69 (0.4-8)	1.76 (1.2-3.7)	10.0	0.5 (0.3-2.5)	0.6 (0.3-3.5)	0.86
ALP (U/L)	186 (114-400)	117 (130-339)	0.77	162 (3.9-212)	117 (59-205)	0.81	224 (63-648)	211 (163-703)	0.64
AST (U/L)	75 (33-100)	44.50 (48-128)	0.36	61 (20-79)	44.50 (25-167)	0.52	137 (59-197)	77.5 (25.5-66)	0.33
ALT (U/L)	34 (18.7-67.2)	37.5 (23.8-53)	0.58	18 (10-35)	31 (17.2-37.7)	0.28	37 (19.4-85.5)	36 (2.7-3.2)	0.81
Albumin (mg/dL)	2.7 (2.4-2.9)	3 (2.5-3.2)	0.11	2.9 (2.6-3.4)	2.8 (2.5-3.5)	0.85	3 (2.3-3.6)	2.9 (27-52)	0.94
Serum creatinine (mg/dL)	1.80 (1.4-1)	1.55 (1.0-4.2)	0.82	1.85 (1.1-4.0)	1.3 (0.8-2.9)	0.20	1.5 (30-80.7)	1 (0.8-2.5)	0.13
BUN (mg/dL)	45.5 (25-72)	42 (22-62)	0.73	55 (33-112)	37.5 (27.2-84.7)	0.22	61 (0.9-3.4)	43 (27-52)	0.27
Procalcitonin (ng/mL)	5.21 (1.3-11.1)	2.67 (1.3-28.7)	0.97	18.36 (3.3-31.7)	2.45 (0.4-13.2)	0.23	2.6 (0.4-38.2)	0.98 (0.4-4.3)	0.32
CK (U/L)	70 (60-77)	66 (60-79)	0.85	75 (69-81)	69 (59-77)	0.19	73 (59-77)	66 (57-79)	0.41

Data measurements are in median (IQR). WBC: White cell count; PT: Prothrombin time; APTT: Activated partial thromboplastin time; ALP: Alkaline liver phosphatase; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; BUN: Blood urea nitrogen; CK: Creatine kinase; IQR: Interquartile range

whole blood cell, platelet, prothrombin time, activated partial thromboplastin time, liver function, Scr, and BUN were all comparable between statin and placebo on D1 and did not differ significantly on D4 and D7 either [Table 2].

Physiological parameters were as depicted in Table 3. Hemodynamics, vasopressor and inotrope dose, perfusion, and metabolic parameters were comparable at start and during the trial week [Table 3]. However, on D7, the maximum noradrenaline dose in statin group was much higher than any other day, albeit nonsignificantly. Furthermore, the lactate levels were neither raised at baseline or during the trial week, nor did they differ significantly between groups. The atorvastatin group also received higher dose (mg/day) of diuretics (30 [20–275] vs. 15 [10–30] in placebo; $P = 0.04$) and consequently also had lower fluid balance on D1.

Inflammatory biomarkers

IL-1, IL-6, TNF- α , IFN, and CRP, all showed a significant decreasing trend, during the trial week, in atorvastatin group as against the placebo group. However, these differences were

comparable on D1, D4, and D7. The delta (Δ) biomarker levels differed significantly [Table 4]. The Δ 1–4 for CRP ($P = 0.002$), Δ 1–7 for all biomarkers, and Δ 4–7 for IL-1 ($P < 0.001$), TNF- α ($P < 0.001$), and CRP ($P = 0.004$) differed significantly. IL-6 was insignificantly higher at baseline in the atorvastatin group.

Lipid profile

Atorvastatin group had insignificantly lower lipid profile levels both before and during the trial week as compared to the placebo group. Furthermore, these and the day-wise Δ lipid profile were also observed to be statistically insignificant [Table 5].

Outcome

There was no significant difference in primary outcome of mortality at D28 after inclusion in study as calculated

Table 3: Day-wise comparison of physiological indices between groups

Variables (physiological)	Day 1		P	Day 4		P	Day 7		P
	Statin group (n=36)	Placebo group (n=37)		Statin group (n=36)	Placebo group (n=37)		Statin group (n=36)	Placebo group (n=37)	
Heart rate (bpm)									
Minimum	97 (83-106)	90 (80-110)	0.68	94 (80-103)	96 (83-110)	0.54	100 (81-110)	99 (82-110)	0.79
Maximum	116 (105-125)	120 (109-134)	0.45	115 (106-131)	118 (110-130)	0.98	112 (100-130)	122 (100-137)	0.59
MAP (mmHg)									
Minimum	75 (70-81)	75 (70-80)	0.74	80 (74-86)	75 (70-81)	0.06	75 (70-84)	75 (70-81)	0.62
Maximum	94.5 (85-103)	100 (90-109)	0.14	95 (89-100)	96 (90-105)	0.74	94.5 (87-107)	98 (81-110)	0.90
NADR (μ g/kg/min)									
Minimum	0.12 (0.06-0.20)	0.10 (0.03-0.30)	0.61	0.10 (0.04-0.37)	0.08 (0.05-0.35)	0.92	0.17 (0.05-1.02)	0.10 (0.04-0.20)	0.19
Maximum	0.30 (0.15-0.50)	0.27 (0.10-0.57)	0.91	0.22 (0.12-0.67)	0.26 (0.09-0.95)	0.82	0.70 (0.18-1.12)	0.20 (0.06-0.65)	0.05
AVP (U/h)	2.4 (2.4-2.4)	2.4 (2.4-2.4)	10.0	2.4 (2.4-2.4)	2.4 (2.4-2.4)	10.0	2.4 (2.4-2.4)	2.4 (2.4-2.4)	10.0
Dobut (μ g/kg/min)	5 (2.5-5)	2.5 (2.5-7.5)	0.74	5 (2.5-5)	5 (3-7.5)	0.21	5 (2.5-5)	3.5 (3-7.5)	0.48
CVO ₂ (%)	76 (70-82)	71 (68-76)	0.06	74 (68-80)	75 (68-80)	0.67	74 (62-81)	76 (72-81)	0.42
Lactate (mg/dL)	12.7 (11.6-16.6)	13.6 (9.8-20)	0.59	13 (9.6-16.2)	14.8 (9.1-24)	0.22	11.9 (8-25)	12 (9.5-17)	0.81
Urine output (mL/day)	1600 (505-2055)	820 (325-1775)	0.17	1715 (945-2685)	1897 (452-2259)	0.96	1450 (600-2195)	1742 (644-2289)	0.68
Diuretic (mg/day)	30 (20-275)	15 (10-30)	0.04*	35 (12-242)	20 (10-110)	0.45	20 (12-242)	20 (10-110)	0.49
CFB (mL/day)	413 (121-1329)	616 (291-1352)	0.18	653 (287-1122)	580 (259-1184)	0.43	509 (251-931)	734 (260-1457)	0.64
pH									
Minimum	7.34 (7.24-7.40)	7.31 (7.22-7.38)	0.53	7.35 (7.30-7.40)	7.35 (7.27-7.41)	0.93	7.33 (7.30-7.40)	7.35 (7.28-7.41)	0.34
Maximum	7.40 (7.36-7.46)	7.40 (7.33-7.45)	0.61	7.41 (7.38-7.46)	7.43 (7.35-7.45)	0.12	7.40 (7.38-7.46)	7.40 (7.35-7.45)	0.93
PaCO ₂ mmHg									
Minimum	38.5 (32-45)	38 (33-42)	0.91	37.5 (31-47)	39 (35-45)	0.57	37.5 (33-47)	38 (35-45)	0.48
Maximum	44.5 (36-56)	45 (39-55)	0.79	43 (39-50)	45 (38-49)	0.86	42 (39-50)	42 (38-49)	0.89
PaO ₂ /FiO ₂ ratio									
Minimum	195 (120-250)	174 (112-243)	0.51	245 (181-300)	200 (159-250)	0.11	222 (181-300)	222 (159-250)	0.61
Maximum	250 (201-345)	235 (190-320)	0.47	300 (226-389)	280 (200-300)	0.11	300 (226-389)	280 (200-300)	0.53
Ventilator support (cmH ₂ O)									
PS	18 (16-22)	18 (15-20)	0.35	19 (15-22)	18 (15-20)	0.95	18 (15-22)	18 (15-20)	0.51
PEEP	10 (8-10)	10 (8-12)	0.47	8 (7-10)	10 (8-12)	0.20	8 (7-10)	8 (8-12)	0.56

All measurements are in median and IQR. * $P < 0.05$; mode of mechanical ventilation was SIMV (pressure control) with pressure support. MAP: Mean arterial pressure; NADR: Noradrenaline; AVP: Arginine vasopressin; CVO₂: Central venous oxygen saturation; FB: Fluid balance; PS: Pressure support; PEEP: Positive end expiratory pressure; IQR: Interquartile range; SIMV: Synchronized Intermittent Mandatory Ventilation

Table 4: Day-wise comparison of trends in biomarkers between groups

Biomarkers	Atorvastatin (n=36) Placebo (n=37)	D1	D4	D7	P#	Δ1-4##	Δ1-7##	Δ4-7##
IL-1 (pg/mL)	Statin	29.34 (3.80-96.50)	8.84 (3.80-47.24)	3.8 (3.8-16.37)	<0.001*	0.12	0.001*	<0.001*
	Placebo	26.46 (3.8-68.98)	3.8 (14.44-76.43)	3.8 (3.80-93.01)	0.48	0.99	0.47	0.42
	P	0.62	0.69	0.15				
IL-6 (pg/mL)	Statin	54.98 (5.99-273.60)	38.82 (4.60-131.19)	8.87 (4.60-91.07)	0.04*	0.74	0.02*	0.09
	Placebo	16.00 (4.60-84.66)	35.13 (4.60-130.35)	53.38 (4.6-91.07)	0.88	0.41	0.23	0.54
	P	0.16	0.74	0.41				
TNF-α (pg/mL)	Statin	42.97 (25.98-66.04)	37.84 (31.11-63.16)	30.15 (17.01-41.04)	0.001*	0.39	<0.001*	<0.001*
	Placebo	35.59 (25.82-59.47)	35.27 (22.61-53.70)	26.94 (12.36-50.66)	0.37	0.67	0.44	0.87
	P	0.25	0.22	0.42				
IFN (pg/mL)	Statin	4.6 (4.6-4.80)	4.4 (4.4-4.80)	4.2 (4.0-4.80)	0.01*	0.92	0.008*	0.06
	Placebo	4.6 (4.6-6.25)	4.6 (4.6-10.98)	4.6 (4.6-4.60)	0.39	1.0	0.58	0.85
	P	0.44	0.89	0.89				
CRP (mg/dL)	Statin	12 (9.50-19.70)	7.33 (3.87-11.36)	5.20 (1.78-7.14)	0.002*	0.03*	0.002*	0.004*
	Placebo	12.3 (7.50-19.70)	12.3 (9.14-13.60)	11.73 (9.90-13.10)	0.65	0.58	0.05	0.41
	P	0.94	0.93	0.01*				

Data measurements are in median IQR; * $P < 0.05$; P: Mann-Whitney U-test; #P: Friedman ANOVA test; ##P: Wilcoxon signed ranks test. IL: Interleukin; TNF-α: Tumor-necrosis-factor alpha; IFN: Interferon; CRP: C-reactive protein; Δ: Delta; IQR: Interquartile range; ANOVA: Analysis of variance

Table 5: Day-wise comparison of trends in lipid profile between groups

Lipid profile (mg/dL)	Atorvastatin (n=36) Placebo (n=37)	D1	D4	D7	P#	Δ1-4##	Δ1-7##	Δ4-7##
TC	Statin	78 (64.5-117.0)	93 (73.0-97.5)	83 (73.0-96.0)	0.81	0.55	0.49	0.73
	Placebo	119 (98.5)	124 (92.0-257.5)	90 (88.2-139.0)	0.18	0.84	0.16	0.31
	P	0.12	0.04*	0.10				
HDL	Statin	42 (36.5-47.5)	42 (34.5-48.0)	41 (37.5-45.5)	0.57	0.85	0.41	0.41
	Placebo	45 (41.5-48.5)	45 (42.2-49.7)	47.50 (41.0-50.5)	0.57	1.0	1.0	0.41
	P	0.25	0.41	0.13				
LDL	Statin	85 (68.0-92.5)	87 (75.0-88.5)	84 (79.0-87.5)	0.57	0.88	0.90	0.44
	Placebo	90.50 (83.0-106.2)	95.50 (84.5-118.0)	85 (66.0-91.7)	0.06	1.0	0.07	0.07
	P	0.16	0.09	0.51				
VLDL	Statin	9 (6.6-24.4)	13.60 (10.5-21.9)	10.80 (9.4-21.0)	0.81	0.77	0.67	0.65
	Placebo	24.20 (16.2-46.8)	26.20 (15.4-41.2)	16.90 (12.2-27.4)	0.96	1.0	0.31	0.44
	P	0.06	0.04*	0.05				
TG	Statin	45 (33.5-122.0)	68 (53.0-110.0)	54 (46.0-105.0)	0.97	0.63	0.77	0.57
	Placebo	127 (81.2-488.2)	131.50 (76.5-206.0)	84 (61.0-137.2)	0.43	0.56	0.06	0.44
	P	0.04*	0.04*	0.05				

Data measurements are in median IQR; * $P < 0.05$; P: Mann-Whitney U-test; #P: Friedman ANOVA test; ##Δ: Wilcoxon signed ranks test. TC: Total cholesterol; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; VLDL: Very low density lipoprotein; TG: Triglyceride; Δ: Delta; IQR: Interquartile range; ANOVA: Analysis of variance

by Kaplan-Meier estimate and compared by application of the log-rank [Figure 2]. Secondary outcomes estimated by event-free days and length of ICU stay were also comparable [Table 6].

Adverse effects

Serial CK levels did not differ significantly between groups before and during trial week [Table 2]. None of the patients in atorvastatin group had a rise of CK ≥ 10 times the normal value. ALT levels also did not rise significantly in the atorvastatin group. Furthermore, no deterioration in renal profile was observed with atorvastatin.

DISCUSSION

Mortality in septic shock remains high despite advances in management. Pleiotropic impact of statins, discordance between observational and randomized studies, variability among existing RCTs, and limited inclusion of septic shock patients was the stimulus for this RCT. The main findings from our study were that 40 mg of atorvastatin for 7 days at onset of septic shock did not decrease mortality or vasopressor-, ventilation-, RRT-free days. However, it did result in decreased levels of inflammatory biomarkers, IL-1, IL-6, TNF-α, IFN, and CRP levels without any detectable drug-related adverse effects or toxicities.

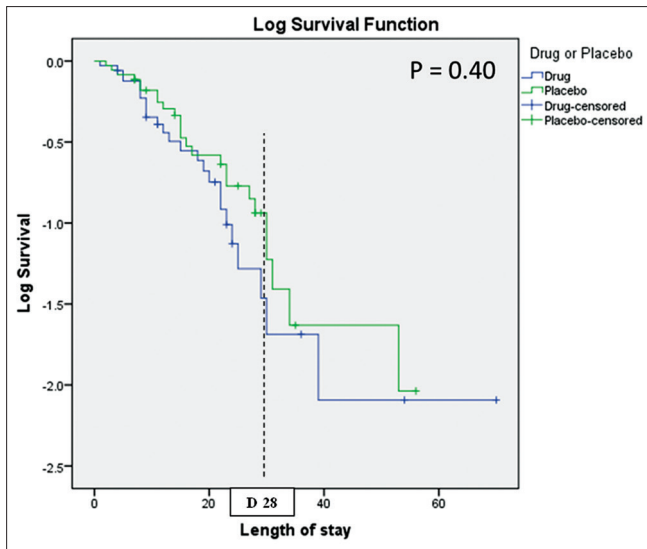


Figure 2: Kaplan–Meier survival analysis

Atorvastatin is the most widely prescribed statin with well-established clinical safety profile even at variable strengths and duration of use. Previous studies of statins in sepsis have used both higher and lower doses of same or different statins for a longer duration than in our study. An unacceptably high mortality of 30%–70% was observed in a retrospective audit of septic shock patients admitted to our ICU (not reported). Length of ICU stay was also observed to be very unpredictable. Fear of adverse drug effects or toxicities, multiple drug interactions, multiorgan dysfunction/failure in septic shock, nonavailability of therapeutic drug monitoring in our setting, and already available negative previous studies of statins in sepsis wherein smaller doses for longer duration had been used prompted us to use higher atorvastatin dose but for a shorter duration.

In spite of the profound variability seen in previous RCTs, mortality benefit of statins in sepsis has been universally rejected by all. A table of previous double-blinded RCTs of statins in sepsis is provided in the Supplementary File. Varying proportions (0%–79%) of septic shock patients have been included in previous RCTs.^[10–16] The present RCT, an exclusive cohort of septic shock patients using atorvastatin (higher dose albeit shorter course), is in concordance with previous RCTs.

Significant earlier hemodynamic stabilization at both lower dose and shorter duration of vasopressor was reportedly achieved with rosuvastatin in the study by El Gendy and Elsharnouby.^[15] In contrast to this, we did not observe any significant difference in either hemodynamics or vasopressor dose or vasopressor-free days between statin and placebo group. The reasons for these differences could be multifactorial. Inclusion of nonseptic shock patients, severity of illness, source of sepsis, coexisting illness, fluid status, choice of antimicrobials, and timing of onset of sepsis and start of therapy may all be plausible explanations. A higher dose of noradrenaline ≥ 0.7 (0.18–1.12) was used specifically

Table 6: Comparison of outcomes between groups in septic shock patients

Patient characteristics	All patients (n=73)	Atorvastatin group (n=36)	Placebo group (n=37)	P
Mechanical lung ventilation, n (%)				
D1	70 (96)	33 (92)	37 (100)	0.30
D4	67 (92)	33 (92)	34 (92)	0.69
D7	51 (70)	28 (78)	23 (62)	0.60
Renal replacement therapy, n (%)				
D1	19 (26)	8 (22)	11 (30)	0.38
D4	12 (16)	6 (17)	6 (16)	0.96
D7	10 (14)	4 (11)	6 (16)	0.39
Rate of RRT	3 (1-6)	3 (1-5)	3 (2-6)	0.65
Event free days				
Vasopressor	3 (0-10)	2 (0-11)	3 (0-8)	0.85
Ventilation	0 (0-2)	0	0 (0-5)	0.83
RRT	10 (6-20)	10 (7-18)	12 (4-25)	0.89
Length of ICU stay, days				
Total stay	16 (9-28)	15 (9-24)	17 (10-28)	0.34
After inclusion in study	14 (7-28)	14 (8-26)	15 (6-28)	0.75
D28 mortality, n (%)	42 (57)	21 (58)	21 (57)	0.89

All measurements are in median IQR unless specified. RRT: Renal replacement therapy; ICU: Intensive Care Unit; IQR: Interquartile range

in 10 patients in the statin group on D7 in our study [Table 3]. Higher severity of illness and nonsurvival explains the higher vasopressor requirements in this select group of patients. Truwit *et al.* in their study on sepsis-associated acute respiratory distress syndrome had reported that rosuvastatin group had lesser free days of ventilation, cardiovascular, coagulation, and renal and hepatic failure.^[16] However, only renal and hepatic failure-free days were significantly lesser in the statin group. Similarly, in our study too, there were insignificantly lesser free days of vasopressor, ventilation, and RRT in the atorvastatin group.

Three out of seven RCTs done previously reported levels of IL-6^[10,11,13] and one reported both IL-6 and TNF- α ^[10] to assess the impact of statins. Novack *et al.* in a single-center RCT reported significant reduction in IL-6 and TNF- α after 72 h of 40 mg simvastatin in patients of suspected or documented bacterial infection not in severe sepsis.^[10] Similarly, Kruger *et al.* in single-center study in 2011 reported a decreasing trend in IL-6 over time albeit insignificantly.^[11] However, the same author in a multicenter study from 21 ICUs in 2013 reported no difference in IL-6 levels between study and placebo group.^[13] Our study has utilized a more comprehensive inflammatory panel of IL-1, IL-6, TNF- α , IFN, and CRP to assess the impact of statins in septic shock. It is unclear why the atorvastatin group had a somewhat higher IL-6 levels upon enrollment even though the groups were otherwise equally matched [Table 4]. Given the small sample size, a chance

occurrence is a plausible explanation. Biomarker levels, comparable at baseline, reduced significantly in all by D7 in the atorvastatin group. These variations are suggestive of the impact of atorvastatin on inflammatory biomarkers in septic shock in spite of being used for a brief period of 1 week. Potential sustenance of this effect cannot be discussed as biomarker estimations were not performed beyond the trial week. However, the positive effects in biomarkers were not replicated in laboratory or physiological parameters both during the trial week [Tables 2 and 3] and during the latter 3 weeks of observation (table not shown). Plausible reason for this discordance could be that the drug dose was just enough to alter inflammatory biomarker levels but of inadequate duration to produce any clinically meaningful effect on physiological or laboratory parameters.

In the absence of plasma statin levels, Patel *et al.* suggested reduction in plasma lipids as indicative of treatment compliance, absorption of statins, and surrogate marker of their pleiotropic effects.^[12] Two previous RCTs studied the effect of statins on lipid profile with differing results. The study by Patel *et al.*^[12] reported significant reduction in lipid profile (TC and LDL) after 40 mg of atorvastatin on D4, while Kruger *et al.*^[13] reported no difference with use of 20 mg atorvastatin. In our study, we observed insignificant difference between groups at baseline or over time [Table 5] somewhat similar to Kruger *et al.*^[13] However, our study in no-way explains a different effect with differing doses and duration of statins in septic shock. In the absence of plasma statin levels, the next best ideal parameter to assess the impact of statins (lipid profile vs. inflammatory biomarkers) is not yet clear.

Similar to previous RCTs, we did not observe any significant elevation of ALT or CK levels in the study population. In none of the patients, trial drug was withdrawn on account of rise in ALT or CK. Furthermore, no deterioration in renal profile was observed with atorvastatin. Large RCT of rosuvastatin in cardiac surgery patients reported adverse renal impact.^[17] However, cardiac surgery and septic shock patients are dissimilar cohort and the insult in either cannot be equated. Although the difference in class, dose, cohort, and ethnicity can plausibly explain the discordance, caution is still advisable as ours is a small study.

Conflicting results exist for the time course of pleiotropic and lipid-lowering effects. Animal studies report that pleiotropic effects occur earlier and independent of lipid-lowering effects.^[18] However, an editorial on acute effects of statins commented that that the two could occur simultaneously making it difficult to assess the relative contribution.^[19] In our study, the effects of inflammation occurred while no changes were seen in the lipid profile [Tables 4 and 5]. Type, dose, route, timing, and duration of statin use along with severity of illness, drug–drug interaction, and genetic makeup of the patient have important implications in acute effect of statins. Intracellular statin concentrations, kinetics, and role of specific drug transporters are poorly understood as of now.^[19] Oral

application may not be the best option in septic shock, but as of now intravenous formulation is not available.^[19] Benefit of intravenous statins in septic shock needs to be explored.

Our study has several limitations. Small sample size, single-center, absence of plasma atorvastatin levels, and short duration of trial drug are its major limitations. The 5-year audit of our unit revealed that the minimum mortality in our septic shock patients was approximately 30%. Whether or not introduction of statins in our septic shock management protocol would reduce the mortality was the question. The minimum absolute mortality difference between the statin and placebo group that we desired was 30% between groups. To achieve this, a sample size of 28–28 was enough to achieve 80% power with 95% confidence. However, keeping a fall-out rate of 10%–15%, we estimated the sample size of 30 in each group. Hence, the actual sample size of 36 and 37 in the drug and placebo group is larger than actually required. The sample size of our study may look small but is actually not too small to justify the mortality difference. Source of sepsis has a strong impact on prognosis. Larger sample size with subgroup analysis would have helped differentiate the impact of therapy versus disease groups. Patients were received mostly during the postresuscitation phase of septic shock resulting in lower baseline lactate levels. Furthermore, the time of onset of sepsis, septic shock, fluid resuscitation, and admission to ICU did not necessarily occur simultaneously. Lactate is a sensitive, albeit nonspecific, stand-alone indicator of cellular or metabolic stress rather than shock. Higher mortality in our study is worrisome. Some of the reasons for this high mortality are as follows: delayed inter- or intra-hospital transfer of critically ill patients to our ICU from either nonexistent or poor quality ICUs, multisite colonization, inappropriate antimicrobial use, higher prevalence of multidrug resistance, inadequately staffed ICU with nurse–patient ratio of $\geq 1:2$ or $1:3$ on most occasions, nonavailability of round the clock respiratory physiotherapist, inadequate microbiological surveillance, inclusion of only septic shock patients, and nonavailability of advanced cardiovascular support systems like extracorporeal membrane oxygenation. These reasons may make our findings not generalizable to other ICUs.

CONCLUSIONS

In statin naïve septic shock patients, atorvastatin of 40 mg/day for 7 days does impact the levels of IL-1, IL-6, TNF- α , IFN, and CRP. However, these alterations did not confer any mortality or secondary benefit though no adverse effects were seen either. Hence, as of now, evidence from our and previous RCTs questions the utility of oral atorvastatin in septic shock. However, the possibility of a future large study using intravenous statins exists.

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Conflicts of interest

There are no conflicts of interest.

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SUPPLEMENTARY FILE

MANAGEMENT OF SEPTIC SHOCK

An ultrasound-guided central vein catheter, an intra-arterial catheter, an invasive mechanical ventilation, a nasogastric tube, and a urinary catheter were placed in all patients under asepsis as soon as possible after Intensive Care Unit admission.

FLUIDS

Sepsis-induced tissue hypoperfusion defined as hypotension (mean arterial pressure [MAP] <65 mmHg) persisting after initial fluid challenge or blood lactate concentration ≥ 4 mmol/L (18 mg/dL) was managed with fluid resuscitation. The first 6-h goals of resuscitation included central venous pressure of 8–12 mmHg, MAP ≥ 65 mmHg, urine output ≥ 0.5 ml/kg/h, central or mixed venous oxygen saturation of 70% or 65%, respectively, and normalization of lactate. The initial fluid for resuscitation was crystalloids; albumin was used if substantial amount of crystalloid was required. Fluid challenge was guided by dynamic (e.g., pulse pressure or stroke volume variation) or static (e.g., arterial pressure, heart rate) variables.

VASOPRESSORS AND INOTROPES

Noradrenaline was the vasopressor of choice to target MAP 65 mmHg. Vasopressin 0.04 units/min was added to improve the MAP, except in patients with acute coronary ischemia or preexisting coronary artery disease. Dobutamine infusion up to 15 $\mu\text{g}/\text{kg}/\text{min}$ was tried in the presence of myocardial dysfunction as suggested by elevated cardiac filling pressures and low cardiac output or ongoing signs of hypoperfusion

STEROIDS

Continuous infusion of hydrocortisone 200 mg/day was used in septic shock when adequate fluid resuscitation and vasopressor therapy were unable to restore hemodynamic stability. This was tapered over 5–7 days, depending on the response.

BLOOD AND BLOOD PRODUCT TRANSFUSIONS

Red blood cell transfusion was administered when hemoglobin (Hb) decreased to <7 g/dL to target Hb of 7–9 g/dL. Higher Hb was targeted in patients with myocardial ischemia, severe hypoxemia, acute hemorrhage, or ischemic heart disease. Fresh frozen plasma and platelet transfusion were used only when a patient with deranged coagulation or low platelet count was bleeding or invasive procedures were planned. Platelets were prophylactically transfused with counts $<10 \times 10^9/\text{L}$

ANTIMICROBIALS AND SOURCE CONTROL

Clinically appropriate cultures and imaging studies were performed before start of antimicrobial(s). At least two

sets of blood cultures (both aerobic and anaerobic) were obtained before antimicrobial(s). Effective intravenous antimicrobials were aimed to be administered within the 1st h of recognition of septic shock and severe sepsis. Initial empiric therapy with ≥ 1 anti-infective agents was selected based on activity against all likely pathogens (bacterial and/or fungal) and pharmacology. Combination empirical therapy was used for neutropenic patients with severe sepsis and for difficult-to-treat, multidrug-resistant (MDR) bacterial pathogens. Antimicrobial regimen was reassessed daily for potential de-escalation, aided by procalcitonin trends and susceptibility profile. Duration of therapy was typically 5–7 days. However, patients with slow clinical response, undrainable septic foci, MDR bacteremia, and some fungal infections or immunodeficiency, including neutropenia, received a longer course of antimicrobial(s). Emergent source control with least physiologic insult (e.g., percutaneous rather than surgical drainage of an abscess) was sought and undertaken as early as possible. An intravascular access device as a possible source of sepsis was removed promptly.

MECHANICAL VENTILATION

In patients on mechanical ventilation, target tidal volume of ≤ 6 mL/kg predicted body weight and plateau pressure of ≤ 30 cm H₂O was aimed at in sepsis-induced acute respiratory distress syndrome (ARDS). Positive end-expiratory pressure (PEEP) was applied to avoid alveolar collapse. Higher rather than lower level of PEEP, recruitment maneuver, and/or prone positioning was used for patients with severe refractory hypoxemia. Head of bed was elevated to 30°–45° to decrease the risk of aspiration and subsequent ventilator-associated pneumonia. Spontaneous breathing trial (SBT) with pressure support ventilation or T-piece was administered regularly to attempt weaning as and when the patient was conscious, hemodynamically stable, low ventilatory and PEEP requirement, and low FiO₂ requirement which could be safely delivered with a face mask. Successful SBT resulted in consideration for extubation. Conservative rather than liberal fluid strategy was the norm in patients with established sepsis-induced ARDS.

RENAL REPLACEMENT

Continuous renal replacement therapy was used for fluid management in hemodynamically unstable septic acute kidney injury patients with oligoanuria and/or refractory metabolic acidosis or hyperkalemia. Sustained low-efficiency dialysis was the preferred modality except when it was hemodynamically intolerable or continuous anticoagulation not desirable. Sodium bicarbonate was avoided.

SEDATION AND PARALYSIS

Continuous or intermittent sedation was titrated in mechanically ventilated sepsis patients, to a target Richmond Agitation–Sedation Score of 2 or higher. A short course of neuromuscular blocking agent (intermittent bolus or continuous infusion) not exceeding 48 h was used only for patients with early sepsis-induced ARDS and a PaO₂/FiO₂ <150 mmHg.

VENOUS THROMBOEMBOLISM AND STRESS ULCER PROPHYLAXIS

Patients received daily subcutaneous low-molecular-weight heparin and/or intermittent pneumatic compression against venous thromboembolism. Dalteparin was used if creatinine clearance was <30 ml/min. Stress ulcer prophylaxis using proton pump inhibitor was given to all patients with risk factors for bleeding.

NUTRITION AND GLYCEMIC CONTROL

Enteral feeding was initiated in all patients as soon as the hemodynamics allowed. Low-dose feeding (e.g., up to 500 calories per day) was advanced as tolerated. Intravenous glucose and enteral nutrition was preferred to total parenteral nutrition (TPN) alone or in conjunction with enteral feeding. Prokinetics were initiated prophylactically. Continuous infusion feeds instead of bolus feeds were started if gastric residual volume was >50% of enteral intake. Feed intolerance with nasogastric feeds was managed with nasojejunal (NJ) feeds (NJ tube placed in radiology suite under fluoroscopy). A protocol for evaluation of safety, delivery, continuity, and success of enteral nutrition was in place for all patients. If this was also not tolerated, TPN was initiated. Euglycemia (target blood glucose 150–180 mg/dl) was maintained with continuous insulin infusion.

Supplementary Table: Summary of the double-blinded randomized placebo controlled trials of statins in sepsis

First author (reference), year, center, country	Site of study	Statin: Placebo	Patients included in the study	Statin, dose, duration	Results: Primary and secondary outcomes	Strengths and limitations of the study as suggested by author
Novack V, ^[10] 2009, single center, Israel	Medicine ward and ICU	42:41	Age ≥18 years No prior statins in previous 3 months Suspicion/evidence of bacterial infection and on intravenous antibiotics Not in severe sepsis	Simvastatin, 40 mg/days, followed by 20 mg/days until hospital d/s or onset of severe sepsis	Primary Significant ↓ IL-6 and TNF-α in statin group after 72 h of therapy but no difference between groups Secondary No difference in biochemical and hematological profiles or any other major clinical outcomes upon enrollment and during hospitalization	Strengths Important preliminary evidence that statins may indeed affect the inflammatory response in acute bacterial infection Limitations Small sample size and underpowered study Selection bias cannot be excluded Cannot comment on the effects of statins over time and course of the inflammatory response
Kruger P, ^[11] 2011, single center, Australia	General ward and ICU	75:75	Suspicion/evidence of infection and on antibiotics ≥2 SIRS Prior statin therapy	Atorvastatin, 20 mg/days, for 28 days or hospital d/s	Primary No difference in severe sepsis or organ dysfunction over time between groups Secondary Hospital mortality and LOS not different between groups IL-6 ↓ over time with statin, but nonsignificant difference between groups Nonsignificant difference in HDL, TG between groups over time Adverse events No difference in CK, ALT between groups	Strengths First to examine use of continued statin on progression and regression of sepsis and inflammation in hospitalized patients of infection Confirms previous observations of the profound decrease in HDL seen in sepsis Limitations None commented

Contd...

Supplementary Table: Contd...

First author (reference), year, center, country	Site of study	Statin: Placebo	Patients included in the study	Statin, dose, duration	Results: Primary and secondary outcomes	Strengths and limitations of the study as suggested by author
Patel M, ^[12] 2012, single center, England	Ward	49:51	Age >18 years Suspicion/evidence of new infection ≥2 SIRS for <24 h No severe sepsis Statin naïve sepsis hospitalized patients	Atorvastatin, 40 mg/days, for hospital stay or up to a maximum of 28 days	Primary Significantly lower conversion to severe sepsis with statins Secondary No difference in rate of ICU admission or hospital readmission, LOS, and mortality at 28 days or 1 year Lipid profile Significant ↓ TC, LDL in statin group Adverse events None	Strengths First to examine the impact of the acute administration of statins on progression of sepsis in statin-naïve patients Supports previous observational studies that pre-existing statin therapy have better sepsis-related outcomes compared to matched controls 2 nd largest RCT to date and the 1 st to suggest benefit from acute statin therapy Limitations Trial did not achieve its recruitment target The screening process was neither specific nor sensitive for identifying patients with sepsis
Kruger P, ^[13] 2013, multicenter, Australia	21 ICUs	123:127	Critically ill adults Strong suspicion/evidence of infection ≥3 SIRS within 48 h before randomization Organ dysfunction of <24 h	Atorvastatin, 20 mg/days, for 14 d or until death or d/s whichever is earlier Prior statin users (n=77)	Primary outcomes No difference in IL-6 between groups and over time Significant lower IL-6 levels at baseline in prior statin users Secondary outcomes Clinical No difference in ICU, hospital, 28 or 90 days mortality, or LOS Continued atorvastatin in prior statin users showed significant improvement at 28 days but not 90 days Biological No difference in CRP levels between groups or trends over time Lipid profile No difference in TC, HDL, or trends over time between groups Adverse events No difference in ALT, CK levels between groups	Strengths Multicenter study Preliminary evidence of safety of continuing statins in critically ill patients Studied the sickest cohort of patients Used a validated inflammatory marker as the primary end point, and stratified at baseline for prior statin use Demonstrated <i>Pk-Pd</i> effects by measuring plasma atorvastatin concentrations accompanied by ↓ plasma cholesterol Limitations This study was too small and the confidence intervals too large to exclude small but important differences in rates of hepatotoxicity and rhabdomyolysis Baseline difference in age despite randomization Higher and more variable IL-6 levels than previously reported in sepsis Underpowered study

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Supplementary Table: Contd...

First author (reference), year, center, country	Site of study	Statin: Placebo	Patients included in the study	Statin, dose, duration	Results: Primary and secondary outcomes	Strengths and limitations of the study as suggested by author
Papazian L, ^[14] 2013, multicenter, France	26 ICUs	146:138	Adults ≥2 days in ICU if VAP (modified CPIS ≥5 and if quantitative BAL fluid, PTC, or an ETA)	Simvastatin, 60 mg/days, till ICU d/s, death, or 28 days, whichever is early Prior statin users (n=26)	Primary outcomes 28 days mortality not significantly decreased with statins Secondary outcomes No difference in 14 days, 90 days, and ICU mortality rates; days outside ICU between day 1 and 28; mortality rates in the subgroups with definite and probable VAP; number of ventilator free days (after successful weaning) between d1 and both 28 days and 90 days	Strengths Multicenter study VAP diagnosis based on bacteriological confirmation by quantitative cultures 1 st to evaluate adjunctive statin therapy in a specific infection (VAP) Limitations Early trial termination so marginal benefits of statins cannot be commented Only specific for VAP Plasma levels of simvastatin not done
El Gendy, ^[15] 2014, single center, Egypt	ICU	54:54	Sepsis Age >18 years Suspicion/evidence of infection ≥2 SIRS, organ dysfunction, hypoperfusion or hypotension Appropriate antibiotic therapy commenced	Rosuvastatin, 20 mg/days for 14 days	Primary outcomes Significantly ↑ number of acceptable blood pressure and systemic perfusion days achieved in shorter time in statin group Secondary outcomes Significantly ↓ dose/duration of vasopressor, arterial lactate, and ventilation days in statin group Significantly ↑ number of organ dysfunction/failure free days in statin group Adverse events Safe from CK and transaminases elevation	Strengths None commented Limitations Comparison with other statins not done No measurement of plasma rosuvastatin levels
Truwit J, 2014, ^[16] multicenter, US	ICU	379:366	ARDS (<300 PaO ₂ /FiO ₂ ratio) requiring mechanical ventilation <24 h of enrollment Suspicion/evidence of infection with any SIRS Prior statin users (54:55)	Rosuvastatin, 40 mg (load) followed by 20 mg/days, until day 3 after d/s from the ICU or study day 28 or hospital d/s, or death, whichever occurred earlier	Primary Mortality and ventilator free days did not differ significantly between groups Secondary No significant differences between groups in any of the other secondary outcomes Adverse events No significant differences between groups	Strengths Large, multicenter Plasma rosuvastatin levels measured Differs both in the choice of statin and in our requirement for a 48-h washout period for statins in order for patients to be eligible for enrollment Limitations None commented

ICUs: Intensive Care Units; IL: Interleukin; TNF-α: Tumor-necrosis-factor alpha; SIRS: Systemic inflammatory response syndrome; LOS: Length of hospital stay; TG: Triglycerides; HDL: High-density lipoprotein; LDL: Low density lipoprotein; ALT: Alanine aminotransferase; CK: Creatine kinase; TC: Total cholesterol; RCT: Randomized controlled trial; CRP: C-reactive protein; VAP: Ventilator-associated pneumonia; CPIS: Clinical Pulmonary Infection Score; BAL: Bronchoalveolar lavage; PTC: Plugged telescopic catheter; ETA: Endotracheal aspiration; ARDS: Acute respiratory distress syndrome; d/s: Discharge; ↑: Increase; ↓: Decrease