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Research

The effect of simvastatin on inflammatory cytokines in community-acquired pneumonia: a randomised, double-blind, placebo-controlled trial

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ABSTRACT:

Objectives: It has been suggested that statins may have an effect on the modulation of the cytokine cascade and on the outcome of patients with community acquired pneumonia (CAP). The aim of this prospective, randomised, double-blind, placebo-controlled trial was to determine whether statin therapy given to hospitalised patients with CAP since hospital admission improves clinical outcomes and reduces the concentration of inflammatory cytokines.

Setting: A tertiary teaching hospital in Barcelona, Spain.

Participants: Thirty-four patients were randomly assigned and included in an intention-to-treat analysis (19 to the simvastatin group and 15 to the placebo group).

Intervention: Patients were randomly assigned to receive 20 mg of simvastatin or placebo administered in the first 24 hours of hospital admission and once daily thereafter for four days.

Outcome: Primary end-point of this trial was the time from hospital admission to clinical stability. However, the trial was stopped because enrolment was much slower than originally anticipated and the study would not have been completed in a reasonable period of time. In the present study we report the findings regarding the effect of simvastatin on inflammatory cytokines.

Results: The baseline characteristics of the patients and cytokine concentrations at the time of enrolment were similar in the two groups. No significant differences in PaO2/FiO2 (P=.37), C-reactive protein (P=.23), tumor necrosis factor-alpha (P=.58), interleukin-6 (P=.64), and interleukin-10 (P=.61) levels at 48 hours of hospitalisation were found between simvastatin and

placebo groups. Similarly, transaminase and total creatine-kinase levels were similar in the simvastatin and placebo groups at 48 hours of hospitalisation (P=.19, .08 and .53 respectively).

Conclusions: The use of simvastatin, 20 mg once daily for four days, since hospital admission did not reduce the levels of inflammatory cytokines in hospitalised patients with CAP.

Clinical Trial Registration: ISRCTN91327214

Strengths and limitations of this study:

- The treatment was assigned on a random basis.
- Another unique feature of this trial was that it addressed the question of de novo statin use only in hospitalised patients with CAP.
- The trial did not achieve its recruitment target for determining the effects of statins on time to reach clinical stability.
- The exclusion criteria, such as patients receiving certain drugs that are metabolized by the CYP3A4 enzyme system, are limitations to the external validity of the results.

INTRODUCTION

Community-acquired pneumonia (CAP) is one of the most important public health problems worldwide.[1] Although mortality in patients with CAP fell dramatically with the introduction of antibiotics in the 1950s, it has changed very little over the past fifty years. Recent studies have found overall mortality rates of 8% to 15%,[2,3] and mortality in patients with CAP requiring intensive care unit (ICU) admission can be as high as 30% despite prompt and appropriate antibiotic therapy.[4]

The concept of clinical stability is a key component of CAP management. It allows decision-making concerning hospital discharge and treatment length. Physicians are well aware that the evolution of hospitalised patients with CAP within the first days is crucial. In fact, once stability was achieved, clinical deterioration occurred in 1% of cases or fewer.[5,6] Studies have shown that excessive inflammatory response is a major cause of treatment failure and mortality in patients with CAP.[7,8] Therefore, there is a growing interest in identifying drugs that can modulate the inflammatory response in these patients. Recently it has been demonstrated that hydroxymethylglutaryl-CoA (HMG-CoA) reductase inhibitors, commonly known as statins, have immunomodulatory, antioxidative and anticoagulant effects. Experimental studies have shown their effect on the modulation of the cytokine cascade and on the organization of the immunological response to respiratory infection.[9] In addition, most observational studies published to date support the idea that the use of statins may improve the prognosis of CAP.[10-12] However, randomised trials are lacking.

In this study, we hypothesized that statin therapy given to hospitalised patients with CAP would reduce the time to clinical stability and the concentration of inflammatory cytokines. Primary end-point of this trial was the time from hospital admission to clinical stability, as

defined elsewhere.[5] Projected enrolment time was estimated to be 36 months. However, the trial was stopped because enrolment was much slower than originally anticipated and the study would not have been completed in a reasonable period of time. The effect of simvastatin in this regard was inconclusive. In the present study we report the findings regarding the effect of simvastatin on inflammatory cytokines.

MATERIALS AND METHODS

Study Design and Setting

This prospective, randomised, double-blind, placebo-controlled trial was conducted at Hospital Universitari de Bellvitge - IDIBELL, a 700-bed public hospital in Barcelona, Spain, between December 2009 and June 2011. The study was approved by the Hospital Research Ethics Committee (AC099/08) and The Spanish Agency of Medicines and Medical Devices. It was registered at International Standard Randomized Control Trial Registry (ISRCTN91327214) before commencement. Informed consent was obtained from all patients. The trial was conducted in accordance with the Declaration of Helsinki and was reported in agreement with the key methodological items of the CONSORT statement.

Patient Eligibility and Recruitment Process

All patients included in the study were at least 18 years of age, had received a diagnosis of CAP in the emergency department, and had required hospital admission according to the following criteria: patients classified in groups I-III of the Pneumonia Severity Index (PSI)[13] with absolute criteria for hospitalisation (need for oxygen therapy or hemodynamic support, pulmonary cavitation, septic metastasis, lack of response to outpatient antibiotic therapy, uncontrollable vomiting). All patients in groups IV and V of the PSI were also included.

Patients who did not provide prior written consent, who had immunosuppression (HIV/AIDS, solid organ transplant, stem cell transplantation, antineoplastic chemotherapy in the previous 30 days, neutropenia, prior use of corticosteroids or other immunosuppressants), and pregnant women were excluded. Similarly, patients receiving statins, antidepressants, calcium channel blockers, amiodarone, azoles, macrolides, niacin, fibric acid and derivatives, protease

inhibitors, and grapefruit juice were not eligible. Finally, patients who received antibiotic therapy or had been admitted more than 24 hours prior to enrolment were also excluded.

Definitions and Follow-up

CAP was defined as the presence of an infiltrate on chest radiography plus at least two of the following: fever (temperature ≥38.0 °C) or hypothermia (temperature ≤35.0 °C), new cough with or without sputum production, pleuritic chest pain, dyspnea, or altered breath sounds on auscultation. The chest radiograph was interpreted by the infectious disease consultant.

Clinical and laboratory data (demographic characteristics, comorbidities, causative organisms, antibiotic susceptibilities, biochemical analysis, empirical antibiotic therapy, and outcomes) on all patients were collected using a computer-assisted protocol. Patients were seen daily during their hospital stay by one or more of the investigators. Pathogens in blood, normally sterile fluids, sputum and other samples were investigated using standard microbiological procedures. Urine antigen tests were performed for the detection of *Legionella pneumophila* serogroup 1 (Binax-Now; Binax, Portland, ME) and *Streptococcus pneumoniae* (Binax-Now; Binax, Portland, ME). In addition, Real-Time Polymerase Chain Reaction was used for the detection of influenza A and B.

Antibiotic therapy was initiated in the emergency department in accordance with hospital guidelines.

Interventions and Randomization

Patients were randomly assigned to receive 20 mg of simvastatin or placebo, which were administered orally in the first 24 hours of hospital admission and once daily thereafter for four

days. Trial packs of identical capsules were prepared by the hospital pharmacy and contained either simvastatin or matched placebo.

Randomisation was performed by using a computer-generated random code with a block size of 10. The random code was held centrally by the clinical epidemiologist and was delivered directly to the pharmacist in charge of the preparation of the masked capsules. All clinical and study personnel and patients remained blinded to the study group assignment throughout the trial.

End-points

In the present analysis, end-points were serum concentrations of inflammatory cytokines (C-reactive protein, tumor necrosis factor alpha, interleukin-6 and interleukin-10) and partial pressure of arterial O2/fraction of inspired O2 ratio (PaO2/FiO2) at 48 hours after treatment administration. Similarly, aminotransferases and total creatine-kinase were determined at 48 hours after admission to evaluate the potential toxicity of treatment.

To determine the cytokine concentrations, 10 ml of venous blood was obtained within 24 hours of hospital admission and after 48 h. Samples were centrifuged at 4000 rpm for 15 min at room temperature. The serum was separated, divided into aliquots and frozen at -80 C within six hours of extraction. For analysis, serum was thawed and TNF-alpha, IL-6, and IL-10 serum concentrations quantified by INVITROGEN Human ELISA kits (Life Technologies) according to the manufacturer's instructions.

Statistical Analysis

Categorical variables were described using counts and percentages. Continuous variables were expressed as the median and interquartile range. Baseline data between the two study groups

were compared by means of the non-parametric Mann-Whitney U test for continuous data, and by the chi square test for categorical data. For 2 × 2 tables in which cells contained fewer than five observations, Fisher's exact two-tailed test for categorical data was used. The Wilcoxon signed-rank test was used to compare two related measurements. Data for the end-point was analysed on intention-to-treat-analysis. A P value of <.05 was considered statistically significant. All reported P values are two-tailed.

All statistical calculations were performed using the Statistical Package for the Social Sciences (Version SPSS 15.01s) for Windows (SPSS Inc, Chicago, IL. USA).

RESULTS

The screening for inclusion criteria was started in December 2009 and ended in June 2011 due to slow recruitment. Most excluded patients were receiving statins, other drugs that are metabolized by the CYP3A4 enzyme system or antibiotic therapy prior to enrolment. Thirty-four patients were randomly assigned and included in an intention-to-treat analysis for the end-point (19 to the simvastatin group and 15 to the placebo group). Figure 1 shows the study profile. The baseline characteristics of the patients at the time of enrolment were similar in the two groups and are detailed in Table 1. No significant differences between groups were documented in the clinical features and severity of patients, the aetiology of CAP, and the type of empirical antibiotic therapy and the time since hospital admission to antibiotic administration.

Table 2 compares serum cytokine concentrations and PaO2/FiO2 in the two groups. No significant differences in TNF-alpha, IL-6 and IL-10, C-reactive protein and PaO2/FiO2 levels at admission and at 48 hours of hospitalisation were found between simvastatin and placebo. However, there were significant changes in cytokine levels at admission compared with those at 48 hours during hospitalisation in each group (Figure 2).

Transaminase (ALT and AST) and total creatine-kinase levels were similar in the simvastatin and placebo groups at 48 hours of hospitalisation (P=.19, .08 and .53 respectively). One patient (in the simvastatin group) required ICU admission and one patient died (in the placebo group).

DISCUSSION

This is a prospective, randomized, double-blind, placebo-controlled trial that evaluated the use of statins in patients with CAP. We found that the use of 20 of simvastatin once daily for four days in addition to the usual care did not reduce the concentrations of inflammatory cytokines in hospitalised patients with CAP.

No prior randomised study has evaluated the effect of statins on inflammatory cytokine levels or clinically relevant outcome parameters in hospitalised patients with CAP. Observational studies including prior users of the drug have related statin therapy with better outcomes in patients with CAP.[10-12] However, an observational study[14] suggested that the healthy user bias has a significant role as a confounding factor in the results. Certainly, the limitations of studies of this kind do not allow the application of their findings in clinical practice.

In a recent study involving adult intensive care patients with different infections and severe sepsis, the investigators did not find differences in IL-6 concentrations between atorvastatin (20 mg daily) and placebo groups.[15] Importantly, another study did not support a beneficial effect of continuing pre-existing statin therapy (atorvastatin 20 mg daily) on sepsis and inflammatory parameters in patients with presumed infection;[16] no significant differences in IL-6 and C-reactive protein decreases were documented at any follow-up time-point in either study group. However, a randomised study in patients with acute bacterial infections found that statin therapy (40 mg of simvastatin, followed by 20 mg of simvastatin) was associated with a reduction in the levels of inflammatory cytokines.[17] A *post hoc* analysis of the subgroup of 48 patients with pneumonia revealed a significant decrease in IL-6 levels, but not in TNF-alpha levels. Nevertheless, it should be noted that IL-6 levels increased at 72 hours in the placebo

group. Moreover, a randomised trial found that the acute administration of 40 mg of atorvastatin daily in patients with sepsis may prevent sepsis progression.[18] The authors postulated that statins may modulate the pathophysiology of sepsis thereby restoring endothelial integrity and thus blocking one of the mechanisms in the development of multiorgan failure. Notably, inflammatory cytokines were not evaluated. Finally, a recent study documented that rosuvastatin did not improve clinical outcomes in patients with sepsis-associated acute respiratory distress syndrome.[19]

Our study suggests that simvastatin does not exert an effect on inflammatory cytokine levels in hospitalised patients with CAP. We evaluated the change in cytokine concentrations within the patient and between simvastatin and placebo groups. The cytokine concentrations decreased rapidly during the first days of hospital admission in both study groups, and no significant differences were documented at 48 hours in cytokine levels between simvastatin and placebo groups. However, cytokine levels were quantified only at baseline and at 48 h, which limits the assessment of statin effects on the further course of the inflammatory response. In addition, it is possible that higher doses of simvastatin or the use of other statins could have produced different results. A 20-mg dosage was selected to address concerns regarding potential toxicity. Interestingly, in a cecal ligation and perforation model of sepsis in mice, Merx *et al*. documented that anti-inflammatory properties vary between individual statins.[20]

Among the strengths of this study is the fact that the treatment was assigned on a random basis. Another unique feature of this trial was that it addressed the question of *de novo* statin use only in hospitalised patients with CAP. In this regard, a recent study documented major differences in the early status of the immune system in relation to the underlying type of infection

and concluded that therapeutic immunointerventions may be directed by the nature of infection.[21] In addition, importantly, our study demonstrated the safety profile of simvastatin. However, it should be noted that patients receiving certain drugs that are metabolized by the CYP3A4 enzyme system were excluded. Moreover, there are certain limitations that should be acknowledged. Firstly, the trial did not achieve its recruitment target for determining the effects of statins on time to reach clinical stability. Nevertheless, previous studies faced similar recruitment problems when conducting sepsis-related searches. Secondly, only systemic cytokine measurements were performed in our study, and this response might differ from that encountered in the lung. Similarly, biomarkers for evaluating coagulation or cardiovascular dysfunction were not evaluated. Finally, although our study population is representative of patients hospitalised with CAP since the clinical features were similar to those reported in other studies, the exclusion criteria are important limitations to the external validity of the results.

In summary, we found that adding simvastatin, at a dose of 20 mg daily for four days, to the usual treatment of hospitalised patients with CAP did not decrease the inflammatory cytokine levels. Due to the difficulty of recruiting patients without exclusion criteria, multicentre randomized studies are needed to determine the precise role of statins on clinically relevant outcome parameters in patients with this infection.

Competing interests: All authors have no conflicts of interest to disclose.

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Contributors: JC designed the study. DV, CGV, AFS, JD, and FL conducted the patient inclusion, reviewed all cases, collected patient information and compiled the data files. MM and FMR collected, processed and compiled the laboratory data. DV, CGV and AFS performed the statistical analyses. JC, DV and AFS drafted the paper. JD, MM, and FMR contributed to critical revision for important intellectual content. All authors approved the final manuscript. JC and DV are the guarantors.

Ethics approval: The study was approved by the Hospital Research Ethics Committee (AC099/08) and The Spanish Agency of Medicines and Medical Devices.

Data sharing statement: No additional data are available.

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Table 1 - Baseline characteristics according to study groups

	Simvastatin	Placebo	P value
	n=19	n=15	
Demographic data			
Age, median (IQR), years	63 (44.5-79)	76 (45.5-78)	.49
Male sex	14 (73.7)	12 (80)	.66
Current smoker	4 (21.1)	4 (26.7)	.91
Comorbidities*	12 (63.2)	9 (60)	.85
Charlson comorbidities index	1 (0-1.5)	1 (0-1)	.39
Clinical features			
Impaired consciousness	2 (10.5)	2 (13.3)	1
Hypotension	1 (5.3)	2 (13.3)	.57
Hypoxemia	12 (63.2)	9 (60)	.85
Multilobar pneumonia	6 (31.6)	5 (33.3)	.91
Leucocytosis (leukocytes >12 109/L)	14 (73.7)	8 (53.3)	.21

CAP-specific scores			
High-risk PSI classes	8 (42.1)	8 (53.3)	.73
Aetiology[†]			
All	11 (57.9)	11 (73.3)	.47
S. pneumoniae	8 (42.1)	8 (53.3)	.73
H. influenzae	0 (0)	2 (13.3)	.18
Influenza A (H1N1)pdm09	1 (5.3)	1 (6.7)	1
Time to antibiotic administration, median (IQR), hours	5.5 (3-8)	5 (4-7.5)	.81
Treatment at admission			
Corticosteroids	8 (42.1)	4 (26.7)	.47
Beta-lactams	15 (78.9)	12 (80)	1
Quinolones	15 (78.9)	9 (60)	.27

Data are reported as n (%), unless otherwise indicated. Abbreviations: IQR, interquartile range; PSI, pneumonia severity index.

[*]Comorbidities included chronic pulmonary diseases, chronic heart diseases, diabetes mellitus, chronic liver disease, chronic kidney disease, dementia and cerebrovascular

disease.

[†]Other aetiologies in simvastatin group were *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* (one case each one)

Table 2 - Serum cytokine concentrations and PaO2/FiO2 upon enrolment and at 48 hours during hospitalization according to study groups

	Simvastatin n=19	Placebo n=15	P value
Within 24 h of admission			
PaO2/FiO2	276.1 (261-299)	276.3 (243-320)	.90
TNF-alpha (pg/ml)	24 (22.3-61.7)	30.6 (20.5-38)	.96
IL-6 (pg/ml)	700 (171-1908)	362 (239-515)	.91
IL-10 (pg/ml)	8.35 (1.5-38.8)	3.2 (2.4-8.3)	.17
At 48 h during hospitalization			
PaO2/FiO2	300 (285-374)	338.1 (314-401)	.37
CRP (mg/dl)	151.2 (59.5-243.6)	69.4 (27.5-212.2)	.23
TNF-alpha (pg/ml)	19.9 (16.7-40.8)	20.6 (15.8-25.5)	.58
IL-6 (pg/ml)	141 (8-192)	66 (37.5-97)	.64
IL-10 (pg/ml)	1.31 (0.4-3.8)	1.16 (0.45-2.2)	.61

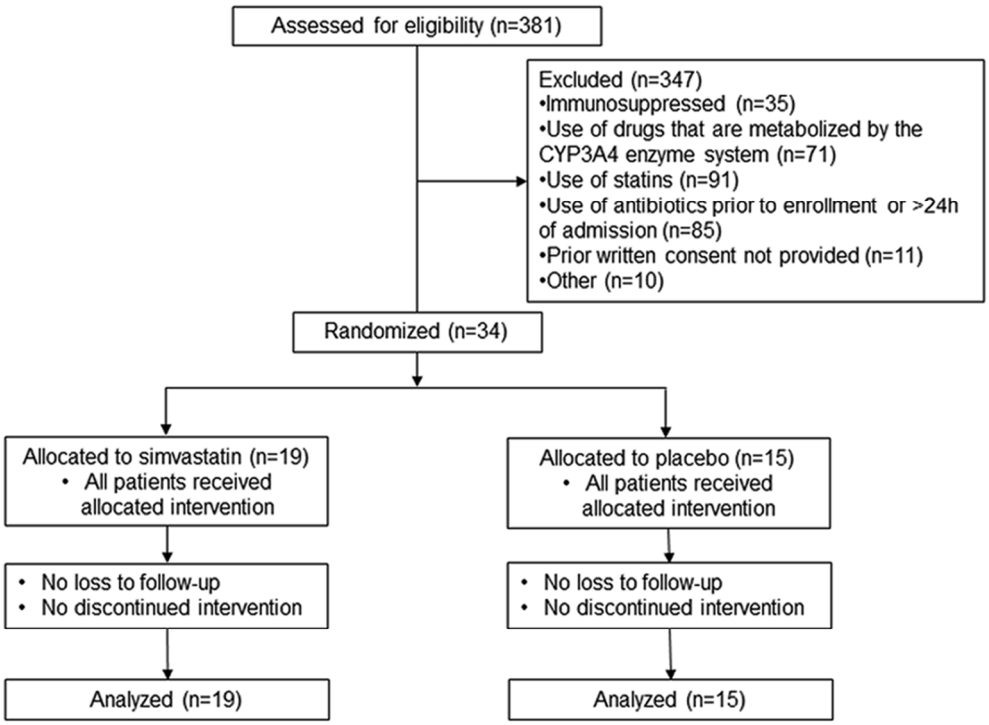
Data are reported as median (interquartile range). Abbreviations: CRP, C-reactive protein;

IL, interleukin; PaO2/FiO2, partial pressure of arterial O2/fraction of inspired O2 ratio; TNF, tumor necrosis factor. CRP levels at baseline were not available.

FIGURE 1. Flowchart of the trial

FIGURE 2. Changes in the cytokine concentrations at admission compared with those at 48 hours during hospitalization in each study group

Concentrations are showed in a logarithmic scale (y axis). All cases P<.001.



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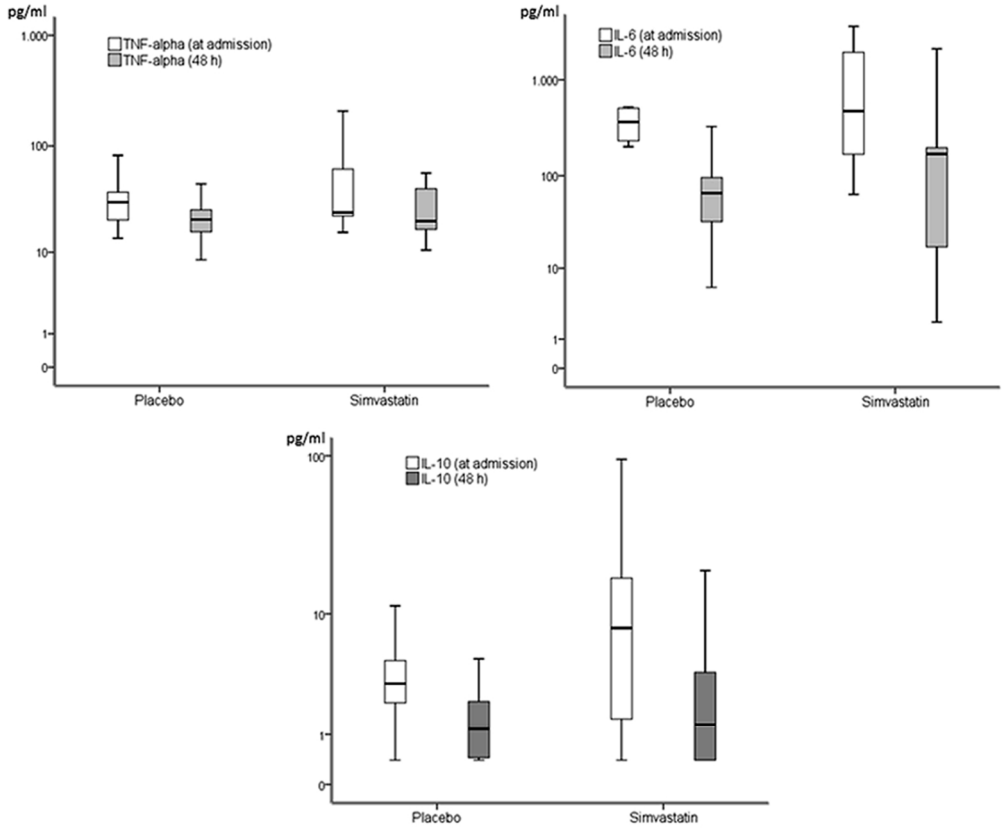

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CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	3
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	6
	2b	Specific objectives or hypotheses	6
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	7,9
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	7
Participants	4a	Eligibility criteria for participants	7
	4b	Settings and locations where the data were collected	7
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	9
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	9
	6b	Any changes to trial outcomes after the trial commenced, with reasons	6,9
Sample size	7a	How sample size was determined	
	7b	When applicable, explanation of any interim analyses and stopping guidelines	
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	9
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	9
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	9
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	9
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	9

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	10
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	11, Figure 1
	13b	For each group, losses and exclusions after randomisation, together with reasons	Figure 1
Recruitment	14a	Dates defining the periods of recruitment and follow-up	11
	14b	Why the trial ended or was stopped	11
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	11, Figure 1
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	11
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	13,14
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	14
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	12,13
Other information			
Registration	23	Registration number and name of trial registry	4
Protocol	24	Where the full trial protocol can be accessed, if available	
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	15

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

Items to include when reporting a randomized trial in a journal or conference abstract

Item	Description	Reported on line number
Title	Identification of the study as randomized	1,3
Authors *	Contact details for the corresponding author	
Trial design	Description of the trial design (e.g. parallel, cluster, non-inferiority)	3
Methods		
Participants	Eligibility criteria for participants and the settings where the data were collected	3
Interventions	Interventions intended for each group	3
Objective	Specific objective or hypothesis	3
Outcome	Clearly defined primary outcome for this report	3
Randomization	How participants were allocated to interventions	3
Blinding (masking)	Whether or not participants, care givers, and those assessing the outcomes were blinded to group assignment	3
Results		
Numbers randomized	Number of participants randomized to each group	3
Recruitment	Trial status	3
Numbers analysed	Number of participants analysed in each group	3
Outcome	For the primary outcome, a result for each group and the estimated effect size and its precision	3
Harms	Important adverse events or side effects	4
Conclusions	General interpretation of the results	4
Trial registration	Registration number and name of trial register	4
Funding	Source of funding	16

**this item is specific to conference abstracts*

BMJ Open

The effect of simvastatin on inflammatory cytokines in community-acquired pneumonia: a randomised, double-blind, placebo-controlled trial

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Research

The effect of simvastatin on inflammatory cytokines in community-acquired pneumonia: a randomised, double-blind, placebo-controlled trial

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ABSTRACT:

Objectives: It has been suggested that statins have an effect on the modulation of the cytokine cascade and on the outcome of patients with community acquired pneumonia (CAP). The aim of this prospective, randomised, double-blind, placebo-controlled trial was to determine whether statin therapy given to hospitalised patients with CAP improves clinical outcomes and reduces the concentration of inflammatory cytokines.

Setting: A tertiary teaching hospital in Barcelona, Spain.

Participants: Thirty-four patients were randomly assigned and included in an intention-to-treat analysis (19 to the simvastatin group and 15 to the placebo group).

Intervention: Patients were randomly assigned to receive 20 mg of simvastatin or placebo administered in the first 24 hours of hospital admission and once daily thereafter for four days.

Outcome: Primary end-point was the time from hospital admission to clinical stability. The secondary end-points were serum concentrations of inflammatory cytokines and PaO2/FiO2 at 48 hours after treatment administration.

Results: The trial was stopped because enrolment was much slower than originally anticipated. The baseline characteristics of the patients and cytokine concentrations at the time of enrolment were similar in the two groups. No significant differences in the time from hospital admission to clinical stability was found between study groups (median 3 days, IQR 2-5 vs 3 days, IQR 2-5; P=.47). No significant differences in PaO2/FiO2 (P=.37), C-reactive protein (P=.23), tumor necrosis factor-alpha (P=.58), interleukin-6 (P=.64), and interleukin-10 (P=.61) levels at 48 hours of hospitalisation were found between simvastatin and placebo groups. Similarly, transaminase

and total creatine-kinase levels were similar between study groups at 48 hours of hospitalisation (P=.19, .08 and .53 respectively).

Conclusions: Our results suggest that the use of simvastatin, 20 mg once daily for four days, since hospital admission did not reduce the time to clinical stability and the levels of inflammatory cytokines in hospitalised patients with CAP.

Clinical Trial Registration: ISRCTN91327214

Strengths and limitations of this study:

- The treatment was assigned on a random basis.
- Another unique feature of this trial was that it addressed the question of de novo statin use only in hospitalised patients with CAP.
- The trial did not achieve its recruitment target for determining the effects of statins on time to reach clinical stability.
- The exclusion criteria, such as patients receiving certain drugs that are metabolized by the CYP3A4 enzyme system, are limitations to the external validity of the results.

INTRODUCTION

Community-acquired pneumonia (CAP) is one of the most important public health problems worldwide.[1] Although mortality in patients with CAP fell dramatically with the introduction of antibiotics in the 1950s, it has changed very little over the past fifty years. Recent studies have found overall mortality rates of 8% to 15%,[2,3] and mortality in patients with CAP requiring intensive care unit (ICU) admission can be as high as 30% despite prompt and appropriate antibiotic therapy.[4]

The concept of clinical stability is a key component of CAP management. It allows decision-making concerning hospital discharge and treatment length. Physicians are well aware that the evolution of hospitalised patients with CAP within the first days is crucial. In fact, once stability was achieved, clinical deterioration occurred in 1% of cases or fewer.[5,6] Studies have shown that excessive inflammatory response is a major cause of treatment failure and mortality in patients with CAP.[7,8] Therefore, there is a growing interest in identifying drugs that can modulate the inflammatory response in these patients. Recently it has been demonstrated that hydroxymethylglutaryl-CoA (HMG-CoA) reductase inhibitors, commonly known as statins, have immunomodulatory, antioxidative and anticoagulant effects. Experimental studies have shown their effect on the modulation of the cytokine cascade and on the organization of the immunological response to respiratory infection.[9] In addition, most observational studies published to date support the idea that the use of statins may improve the prognosis of CAP.[10-12] However, randomised trials are lacking.

In this study, we hypothesized that statin therapy given to hospitalised patients with CAP would reduce the time to clinical stability and the concentration of inflammatory cytokines.

Primary end-point of this trial was the time from hospital admission to clinical stability, as defined elsewhere.[5]

MATERIALS AND METHODS

Study Design and Setting

This prospective, randomised, double-blind, placebo-controlled trial was conducted at Hospital Universitari de Bellvitge - IDIBELL, a 700-bed public hospital in Barcelona, Spain, between December 2009 and June 2011. The study was approved by the Hospital Research Ethics Committee (AC099/08) and The Spanish Agency of Medicines and Medical Devices. It was registered at International Standard Randomized Control Trial Registry (ISRCTN91327214) before commencement. Informed consent was obtained from all patients. The trial was conducted in accordance with the Declaration of Helsinki and was reported in agreement with the key methodological items of the CONSORT statement.

Patient Eligibility and Recruitment Process

All patients included in the study were at least 18 years of age, had received a diagnosis of CAP in the emergency department, and had required hospital admission according to the following criteria: patients classified in groups I-III of the Pneumonia Severity Index (PSI)[13] with absolute criteria for hospitalisation (need for oxygen therapy or hemodynamic support, pulmonary cavitation, septic metastasis, lack of response to outpatient antibiotic therapy, uncontrollable vomiting). All patients in groups IV and V of the PSI were also included.

Patients who did not provide prior written consent, who had immunosuppression (HIV/AIDS, solid organ transplant, stem cell transplantation, antineoplastic chemotherapy in the previous 30 days, neutropenia, prior use of corticosteroids or other immunosuppressants), and pregnant women were excluded. Similarly, patients receiving statins, antidepressants, calcium channel blockers, amiodarone, azoles, macrolides, niacin, fibric acid and derivatives, protease

inhibitors, and grapefruit juice were not eligible. Finally, patients who received antibiotic therapy or had been admitted more than 24 hours prior to enrolment were also excluded.

Definitions and Follow-up

CAP was defined as the presence of an infiltrate on chest radiography plus at least two of the following: fever (temperature ≥38.0 °C) or hypothermia (temperature ≤35.0 °C), new cough with or without sputum production, pleuritic chest pain, dyspnea, or altered breath sounds on auscultation. The chest radiograph was interpreted by the infectious disease consultant.

Clinical and laboratory data (demographic characteristics, comorbidities, causative organisms, antibiotic susceptibilities, biochemical analysis, empirical antibiotic therapy, and outcomes) on all patients were collected using a computer-assisted protocol. Patients were seen daily during their hospital stay by one or more of the investigators. Pathogens in blood, normally sterile fluids, sputum and other samples were investigated using standard microbiological procedures. Urine antigen tests were performed for the detection of *Legionella pneumophila* serogroup 1 (Binax-Now; Binax, Portland, ME) and *Streptococcus pneumoniae* (Binax-Now; Binax, Portland, ME). In addition, Real-Time Polymerase Chain Reaction was used for the detection of influenza A and B.

Antibiotic therapy was initiated in the emergency department in accordance with hospital guidelines.

Interventions and Randomization

Patients were randomly assigned to receive 20 mg of simvastatin or placebo, which were administered orally in the first 24 hours of hospital admission and once daily thereafter for four

days. Four days of simvastatin therapy was chosen because plasma mevalonic acid, substance related with pleiotropic effects, drop up to 70% within 1–2 hours after the first administration of statins, previous studies have administered immunomodulatory therapies between 3 to 7 days, and the median time to clinical stability in our patients is nearly 4 days. Trial packs of identical capsules were prepared by the hospital pharmacy and contained either simvastatin or matched placebo.

Randomisation was performed by using a computer-generated random code with a block size of 10. The random code was held centrally by the clinical epidemiologist and was delivered directly to the pharmacist in charge of the preparation of the masked capsules. All clinical and study personnel and patients remained blinded to the study group assignment throughout the trial.

End-points

Primary end-point of the trial was the time (days) from hospital admission to clinical stability, as described elsewhere.[5] Clinical stability was measured daily during hospitalisation. Secondary end-points were serum concentrations of inflammatory cytokines (C-reactive protein, tumor necrosis factor alpha, interleukin-6 and interleukin-10) and partial pressure of arterial O2/fraction of inspired O2 ratio (PaO2/FiO2) at 48 hours after treatment administration. Similarly, aminotransferases and total creatine-kinase were determined at 48 hours after admission to evaluate the potential toxicity of treatment.

To determine the cytokine concentrations, 10 ml of venous blood was obtained within 24 hours of hospital admission and after 48 h. Samples were centrifuged at 4000 rpm for 15 min at room temperature. The serum was separated, divided into aliquots and frozen at -80 C within six hours of extraction. For analysis, serum was thawed and TNF-alpha, IL-6, and IL-10 serum

concentrations quantified by INVITROGEN Human ELISA kits (Life Technologies) according to the manufacturer's instructions.

Statistical Analysis

In the original analysis, we calculated the sample size on the hypothesis that simvastatin could reduce the time to reach clinical stability by 1.5 days. With a reference time to reach clinical stability of five days, we calculated that 175 patients were needed in each group to detect this difference with a power of 80% and a type 1 error of 5% (two-sided).

Categorical variables were described using counts and percentages. Continuous variables were expressed as the median and interquartile range. Baseline data between the two study groups were compared by means of the non-parametric Mann-Whitney U test for continuous data, and by the chi square test for categorical data. For 2 × 2 tables in which cells contained fewer than five observations, Fisher's exact two-tailed test for categorical data was used. The Wilcoxon signed-rank test was used to compare two related measurements. In addition, analysis of covariance (ANCOVA) was performed to compare the cytokines values at 48 hour in the two groups, adjusting for baseline values.

Data for the end-point was analysed on intention-to-treat-analysis. A P value of <.05 was considered statistically significant. All reported P values are two-tailed. All statistical calculations were performed using the Statistical Package for the Social Sciences (Version SPSS 15.01s) for Windows (SPSS Inc, Chicago, IL. USA).

RESULTS

The screening for inclusion criteria was started in December 2009 and ended in June 2011 due to slow recruitment because of the exclusion criteria of the study. Most excluded patients were receiving statins, other drugs that are metabolized by the CYP3A4 enzyme system or antibiotic therapy prior to enrolment. Thirty-four patients were randomly assigned and included in an intention-to-treat analysis for the end-point (19 to the simvastatin group and 15 to the placebo group). Figure 1 shows the study profile. The baseline characteristics of the patients at the time of enrolment were similar in the two groups and are detailed in Table 1. No significant differences between groups were documented in the clinical features and severity of patients, the aetiology of CAP, and the type of empirical antibiotic therapy and the time since hospital admission to antibiotic administration.

No significant differences in the time from hospital admission to clinical stability was found between study groups (median 3 days, IQR 2-5 vs 3 days, IQR 2-5; P=.47).

Table 2 compares serum cytokine concentrations and PaO2/FiO2 in the two groups. No significant differences in TNF-alpha, IL-6 and IL-10, C-reactive protein and PaO2/FiO2 levels at admission and at 48 hours of hospitalisation were found between simvastatin and placebo. However, there were significant changes in cytokine levels at admission compared with those at 48 hours during hospitalisation in each group (Figure 2). The median change from baseline to 48 hours of PaO2/FiO2 and cytokines between study groups did not differ significantly (Table 3). A *post hoc* subgroup analysis in patients with and without corticosteroids did not found significant differences among cytokines in the study groups (data not shown).

Transaminase (ALT and AST) and total creatine-kinase levels were similar in the simvastatin and placebo groups at 48 hours of hospitalisation (Table 4). One patient (in the simvastatin group) required ICU admission and one patient died (in the placebo group).

DISCUSSION

This is a prospective, randomized, double-blind, placebo-controlled trial that evaluated the use of statins in patients with CAP. We were unable to find that the use of 20 of simvastatin, once daily for four days in addition to the usual care, reduce the time from hospital admission to clinical stability and the concentrations of inflammatory cytokines in hospitalised patients with CAP.

No prior randomised study has evaluated the effect of statins on inflammatory cytokine levels or clinically relevant outcome parameters in hospitalised patients with CAP. Observational studies including prior users of the drug have related statin therapy with better outcomes in patients with CAP.[10-12] However, an observational study[14] suggested that the healthy user bias has a significant role as a confounding factor in the results. Certainly, the limitations of studies of this kind do not allow the application of their findings in clinical practice.

In a recent study involving adult intensive care patients with different infections and severe sepsis (mainly lung, urinary and intraabdominal infections), the investigators did not find differences in IL-6 concentrations between atorvastatin (20 mg daily) and placebo groups.[15] Importantly, another study did not support a beneficial effect of continuing pre-existing statin therapy (atorvastatin 20 mg daily) on sepsis and inflammatory parameters in patients with presumed infection;[16] no significant differences in IL-6 and C-reactive protein decreases were documented at any follow-up time-point in either study group. However, a randomised study in patients with acute bacterial infections found that statin therapy (40 mg of simvastatin, followed by 20 mg of simvastatin) was associated with a reduction in the levels of inflammatory cytokines.[17] A *post hoc* analysis of the subgroup of 48 patients with pneumonia revealed a significant decrease in IL-6 levels, but not in TNF-alpha levels. Nevertheless, it should be noted

that IL-6 levels increased at 72 hours in the placebo group. Moreover, a randomised trial found that the acute administration of 40 mg of atorvastatin daily in patients with sepsis may prevent sepsis progression.[18] The authors postulated that statins may modulate the pathophysiology of sepsis thereby restoring endothelial integrity and thus blocking one of the mechanisms in the development of multiorgan failure. Notably, inflammatory cytokines were not evaluated. Finally, a recent study documented that rosuvastatin did not improve clinical outcomes in patients with sepsis-associated acute respiratory distress syndrome.[19]

Our study suggests that simvastatin does not exert an effect on inflammatory cytokine levels in hospitalised patients with CAP. We evaluated the change in cytokine concentrations within the patient and between simvastatin and placebo groups. The cytokine concentrations decreased rapidly during the first days of hospital admission in both study groups, and no significant differences were documented at 48 hours in cytokine levels between simvastatin and placebo groups. However, cytokine levels were quantified only at baseline and at 48 h, which limits the assessment of statin effects on the further course of the inflammatory response. In addition, it is possible that higher doses of simvastatin[17] or the use of other statins[18] could have produced different results. A 20-mg dosage was selected to address concerns regarding potential toxicity. Interestingly, in a cecal ligation and perforation model of sepsis in mice, Merx *et al*. documented that anti-inflammatory properties vary between individual statins.[20]

Among the strengths of this study is the fact that the treatment was assigned on a random basis. Another unique feature of this trial was that it addressed the question of *de novo* statin use only in hospitalised patients with CAP. In this regard, a recent study documented major differences in the early status of the immune system in relation to the underlying type of infection

and concluded that therapeutic immunointerventions may be directed by the nature of infection.[21] In addition, importantly, our study suggest the safety profile of simvastatin in this context. However, it should be noted that patients receiving certain drugs that are metabolized by the CYP3A4 enzyme system were excluded. This because of the increased risk of rhabdomyolysis during concomitant use of simvastatin, a CYP3A4 substrate statin, and a CYP3A4 inhibitors[22] and legal aspects related to the responsibility insurance of the study.

Moreover, there are certain limitations that should be acknowledged. Firstly, the trial did not achieve its recruitment target for determining the effects of statins on time to reach clinical stability. Nevertheless, previous studies faced similar recruitment problems when conducting sepsis-related searches. Secondly, only systemic cytokine measurements were performed in our study, and this response might differ from that encountered in the lung. Similarly, biomarkers for evaluating coagulation or cardiovascular dysfunction were not evaluated. In addition, other potential benefits of statins in CAP were not assessed in the present study, including stabilization of cardiovascular system to avoid acute cardiac events and their potential antiviral and antibacterial effects. Thirdly, some baseline characteristics, such as corticosteroid use and long time to first antibiotic dose may complicate analysis of cytokine levels. However, no significant differences were observed between study groups regarding these topics. Finally, although our study population is representative of patients hospitalised with CAP since the clinical features were similar to those reported in other studies, the exclusion criteria are important limitations to the external validity of the results. In addition, a clinical trial designed with the exclusion criteria used in the present study is likely difficult, mainly due to recruit adequate number of patients.

In summary, we did not find that adding simvastatin, at a dose of 20 mg daily for four days, to the usual treatment of hospitalised patients with CAP decrease the time from hospital admission to clinical stability and the inflammatory cytokine levels. Due to the difficulty of recruiting patients without exclusion criteria, multicentre randomized studies are needed to determine the precise role of statins on clinically relevant outcome parameters in patients with this infection.

Competing interests: All authors have no conflicts of interest to disclose.

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Contributors: JC designed the study. DV, CGV, AFS, JD, and FL conducted the patient inclusion, reviewed all cases, collected patient information and compiled the data files. MM and FMR collected, processed and compiled the laboratory data. DV, CGV and AFS performed the statistical analyses. JC, DV and AFS drafted the paper. JD, MM, and FMR contributed to critical revision for important intellectual content. All authors approved the final manuscript. JC and DV are the guarantors.

Ethics approval: The study was approved by the Hospital Research Ethics Committee (AC099/08) and The Spanish Agency of Medicines and Medical Devices.

Data sharing statement: No additional data are available.

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Table 1 - Baseline characteristics according to study groups

	Simvastatin	Placebo
	n=19	n=15
Demographic data		
Age, median (IQR), years	63 (44.5-79)	76 (45.5-78)
Male sex	14 (73.7)	12 (80)
Current smoker	4 (21.1)	4 (26.7)
Comorbidities*	12 (63.2)	9 (60)
Charlson comorbidities index	1 (0-1.5)	1 (0-1)
Clinical features		
Impaired consciousness	2 (10.5)	2 (13.3)
Hypotension	1 (5.3)	2 (13.3)
Hypoxemia	12 (63.2)	9 (60)
Multilobar pneumonia	6 (31.6)	5 (33.3)
Leucocytosis (leukocytes >12 109/L)	14 (73.7)	8 (53.3)

IDSA / ATS criteria for ICU Admission[1]	4 (21%)	5 (33.3%)
CAP-specific scores		
High-risk PSI classes	8 (42.1)	8 (53.3)
Aetiology[†]		
All	11 (57.9)	11 (73.3)
S. pneumoniae	8 (42.1)	8 (53.3)
H. influenzae	0 (0)	2 (13.3)
Influenza A (H1N1)pdm09	1 (5.3)	1 (6.7)
Time to antibiotic administration, median (IQR), hours	5.5 (3-8)	5 (4-7.5)
Treatment at admission		
Corticosteroids	8 (42.1)	4 (26.7)
Beta-lactams	15 (78.9)	12 (80)
Quinolones	15 (78.9)	9 (60)
Mechanical ventilation	1 (5.3)	0 (0)

Data are reported as n (%), unless otherwise indicated. Abbreviations: IQR, interquartile range; PSI, pneumonia severity index.

*Comorbidities included chronic pulmonary diseases, chronic heart diseases, diabetes mellitus, chronic liver disease, chronic kidney disease, dementia and cerebrovascular disease.

†Other aetiologies in simvastatin group were *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* (one case each one).

Table 2 - Serum cytokine concentrations and PaO2/FiO2 upon enrolment and at 48 hours during hospitalization according to study groups

	Simvastatin n=19	Placebo n=15	P value
Within 24 h of admission			
PaO2/FiO2	276.1 (261-299)	276.3 (243-320)	.90
TNF-alpha (pg/ml)	24 (22.3-61.7)	30.6 (20.5-38)	.96
IL-6 (pg/ml)	700 (171-1908)	362 (239-515)	.91
IL-10 (pg/ml)	8.35 (1.5-38.8)	3.2 (2.4-8.3)	.17
At 48 h during hospitalization			
PaO2/FiO2*	300 (285-374)	338.1 (314-401)	.37
CRP (mg/dl)	151.2 (59.5-243.6)	69.4 (27.5-212.2)	.23
TNF-alpha (pg/ml)*	19.9 (16.7-40.8)	20.6 (15.8-25.5)	.58
IL-6 (pg/ml)*	141 (8-192)	66 (37.5-97)	.64
IL-10 (pg/ml)*	1.31 (0.4-3.8)	1.16 (0.45-2.2)	.61

* P values for ANCOVA analysis for PaO2/FiO2, TNF-alpha, IL-6 and IL-10 were 0.33,

0.97, 0.31 and 0.55 respectively. Data are reported as median (interquartile range). Abbreviations: CRP, C-reactive protein; IL, interleukin; PaO2/FiO2, partial pressure of arterial O2/fraction of inspired O2 ratio; TNF, tumor necrosis factor. CRP levels at baseline were not available.

Table 3 - Median change of serum cytokine concentrations and PaO2/FiO2 from baseline to 48 hours between study groups

	Simvastatin n=19	Placebo n=15	P value
PaO2/FiO2	-25.4 (0- -68.6)	-64.7 (15.5- -173.3)	.37
TNF-alpha (pg/ml)	5.1 (3.9-14.4)	10.2 (3.2-13.9)	.64
IL-6 (pg/ml)	463 (45.5-1579.5)	354 (169.5-413.5).87	.87
IL-10 (pg/ml)	4.1 (1.1-12.5)	1.8 (0.6-2.9)	.14

Data are reported as median (interquartile range). Abbreviations: IL, interleukin; PaO2/FiO2, partial pressure of arterial O2/fraction of inspired O2 ratio; TNF, tumor necrosis factor.

Table 4 – Adverse events during hospitalization according to study groups

Adverse event	Simvastatin n=19	Placebo n=15	P value
AST level, median (IQR), (ukat/L)	0.25 (0.22-0.36)	0.7 (0.3-0.94)	.08
AST > 2 times upper reference limit	1 (5.2%)	3 (20%)	.30
ALT level, median (IQR), (ukat/L)	0.28 (0.22-0.43)	0.68 (0.3-1.0)	.19
ALT > 2 times upper reference limit	2 (10.5%)	2 (13.3%)	1
CK level, median (IQR), (ukat/L)	0.87 (0.51-2.22)	0.60 (0.32-2.7)	.53
CK > 2 times upper reference limit	1 (5.2%)	1 (6.6%)	1

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; IQR, interquartile range.

FIGURE 1. Flowchart of the trial

FIGURE 2. Changes in the cytokine concentrations at admission compared with those at 48 hours during hospitalization in each study group

Concentrations are showed in a logarithmic scale (y axis). All cases, Wilcoxon signed-rank test P<.001.

Research

The effect of simvastatin on inflammatory cytokines in community-acquired pneumonia: a randomised, double-blind, placebo-controlled trial

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ABSTRACT:

Objectives: It has been suggested that statins have an effect on the modulation of the cytokine cascade and on the outcome of patients with community acquired pneumonia (CAP). The aim of this prospective, randomised, double-blind, placebo-controlled trial was to determine whether statin therapy given to hospitalised patients with CAP improves clinical outcomes and reduces the concentration of inflammatory cytokines.

Setting: A tertiary teaching hospital in Barcelona, Spain.

Participants: Thirty-four patients were randomly assigned and included in an intention-to-treat analysis (19 to the simvastatin group and 15 to the placebo group).

Intervention: Patients were randomly assigned to receive 20 mg of simvastatin or placebo administered in the first 24 hours of hospital admission and once daily thereafter for four days.

Outcome: Primary end-point was the time from hospital admission to clinical stability. The secondary end-points were serum concentrations of inflammatory cytokines and PaO2/FiO2 at 48 hours after treatment administration.

Results: The trial was stopped because enrolment was much slower than originally anticipated. The baseline characteristics of the patients and cytokine concentrations at the time of enrolment were similar in the two groups. No significant differences in the time from hospital admission to clinical stability was found between study groups (median 3 days, IQR 2-5 vs 3 days, IQR 2-5; P=.47). No significant differences in PaO2/FiO2 (P=.37), C-reactive protein (P=.23), tumor necrosis factor-alpha (P=.58), interleukin-6 (P=.64), and interleukin-10 (P=.61) levels at 48 hours of hospitalisation were found between simvastatin and placebo groups. Similarly, transaminase

and total creatine-kinase levels were similar between study groups at 48 hours of hospitalisation (P=.19, .08 and .53 respectively).

Conclusions: Our results suggest that the use of simvastatin, 20 mg once daily for four days, since hospital admission did not reduce the time to clinical stability and the levels of inflammatory cytokines in hospitalised patients with CAP.

Clinical Trial Registration: ISRCTN91327214

Strengths and limitations of this study:

- The treatment was assigned on a random basis.
- Another unique feature of this trial was that it addressed the question of de novo statin use only in hospitalised patients with CAP.
- The trial did not achieve its recruitment target for determining the effects of statins on time to reach clinical stability.
- The exclusion criteria, such as patients receiving certain drugs that are metabolized by the CYP3A4 enzyme system, are limitations to the external validity of the results.

INTRODUCTION

Community-acquired pneumonia (CAP) is one of the most important public health problems worldwide.[1] Although mortality in patients with CAP fell dramatically with the introduction of antibiotics in the 1950s, it has changed very little over the past fifty years. Recent studies have found overall mortality rates of 8% to 15%,[2,3] and mortality in patients with CAP requiring intensive care unit (ICU) admission can be as high as 30% despite prompt and appropriate antibiotic therapy.[4]

The concept of clinical stability is a key component of CAP management. It allows decision-making concerning hospital discharge and treatment length. Physicians are well aware that the evolution of hospitalised patients with CAP within the first days is crucial. In fact, once stability was achieved, clinical deterioration occurred in 1% of cases or fewer.[5,6] Studies have shown that excessive inflammatory response is a major cause of treatment failure and mortality in patients with CAP.[7,8] Therefore, there is a growing interest in identifying drugs that can modulate the inflammatory response in these patients. Recently it has been demonstrated that hydroxymethylglutaryl-CoA (HMG-CoA) reductase inhibitors, commonly known as statins, have immunomodulatory, antioxidative and anticoagulant effects. Experimental studies have shown their effect on the modulation of the cytokine cascade and on the organization of the immunological response to respiratory infection.[9] In addition, most observational studies published to date support the idea that the use of statins may improve the prognosis of CAP.[10-12] However, randomised trials are lacking.

In this study, we hypothesized that statin therapy given to hospitalised patients with CAP would reduce the time to clinical stability and the concentration of inflammatory cytokines.

Primary end-point of this trial was the time from hospital admission to clinical stability, as defined elsewhere.[5]

MATERIALS AND METHODS

Study Design and Setting

This prospective, randomised, double-blind, placebo-controlled trial was conducted at Hospital Universitari de Bellvitge - IDIBELL, a 700-bed public hospital in Barcelona, Spain, between December 2009 and June 2011. The study was approved by the Hospital Research Ethics Committee (AC099/08) and The Spanish Agency of Medicines and Medical Devices. It was registered at International Standard Randomized Control Trial Registry (ISRCTN91327214) before commencement. Informed consent was obtained from all patients. The trial was conducted in accordance with the Declaration of Helsinki and was reported in agreement with the key methodological items of the CONSORT statement.

Patient Eligibility and Recruitment Process

All patients included in the study were at least 18 years of age, had received a diagnosis of CAP in the emergency department, and had required hospital admission according to the following criteria: patients classified in groups I-III of the Pneumonia Severity Index (PSI)[13] with absolute criteria for hospitalisation (need for oxygen therapy or hemodynamic support, pulmonary cavitation, septic metastasis, lack of response to outpatient antibiotic therapy, uncontrollable vomiting). All patients in groups IV and V of the PSI were also included.

Patients who did not provide prior written consent, who had immunosuppression (HIV/AIDS, solid organ transplant, stem cell transplantation, antineoplastic chemotherapy in the previous 30 days, neutropenia, prior use of corticosteroids or other immunosuppressants), and pregnant women were excluded. Similarly, patients receiving statins, antidepressants, calcium channel blockers, amiodarone, azoles, macrolides, niacin, fibric acid and derivatives, protease

inhibitors, and grapefruit juice were not eligible. Finally, patients who received antibiotic therapy or had been admitted more than 24 hours prior to enrolment were also excluded.

Definitions and Follow-up

CAP was defined as the presence of an infiltrate on chest radiography plus at least two of the following: fever (temperature ≥38.0 °C) or hypothermia (temperature ≤35.0 °C), new cough with or without sputum production, pleuritic chest pain, dyspnea, or altered breath sounds on auscultation. The chest radiograph was interpreted by the infectious disease consultant.

Clinical and laboratory data (demographic characteristics, comorbidities, causative organisms, antibiotic susceptibilities, biochemical analysis, empirical antibiotic therapy, and outcomes) on all patients were collected using a computer-assisted protocol. Patients were seen daily during their hospital stay by one or more of the investigators. Pathogens in blood, normally sterile fluids, sputum and other samples were investigated using standard microbiological procedures. Urine antigen tests were performed for the detection of *Legionella pneumophila* serogroup 1 (Binax-Now; Binax, Portland, ME) and *Streptococcus pneumoniae* (Binax-Now; Binax, Portland, ME). In addition, Real-Time Polymerase Chain Reaction was used for the detection of influenza A and B.

Antibiotic therapy was initiated in the emergency department in accordance with hospital guidelines.

Interventions and Randomization

Patients were randomly assigned to receive 20 mg of simvastatin or placebo, which were administered orally in the first 24 hours of hospital admission and once daily thereafter for four

days. Four days of simvastatin therapy was chosen because plasma mevalonic acid, substance related with pleiotropic effects, drop up to 70% within 1–2 hours after the first administration of statins, previous studies have administered immunomodulatory therapies between 3 to 7 days, and the median time to clinical stability in our patients is nearly 4 days. Trial packs of identical capsules were prepared by the hospital pharmacy and contained either simvastatin or matched placebo.

Randomisation was performed by using a computer-generated random code with a block size of 10. The random code was held centrally by the clinical epidemiologist and was delivered directly to the pharmacist in charge of the preparation of the masked capsules. All clinical and study personnel and patients remained blinded to the study group assignment throughout the trial.

End-points

Primary end-point of the trial was the time (days) from hospital admission to clinical stability, as described elsewhere.[5] Clinical stability was measured daily during hospitalisation. Secondary end-points were serum concentrations of inflammatory cytokines (C-reactive protein, tumor necrosis factor alpha, interleukin-6 and interleukin-10) and partial pressure of arterial O2/fraction of inspired O2 ratio (PaO2/FiO2) at 48 hours after treatment administration. Similarly, aminotransferases and total creatine-kinase were determined at 48 hours after admission to evaluate the potential toxicity of treatment.

To determine the cytokine concentrations, 10 ml of venous blood was obtained within 24 hours of hospital admission and after 48 h. Samples were centrifuged at 4000 rpm for 15 min at room temperature. The serum was separated, divided into aliquots and frozen at -80 C within six hours of extraction. For analysis, serum was thawed and TNF-alpha, IL-6, and IL-10 serum

concentrations quantified by INVITROGEN Human ELISA kits (Life Technologies) according to the manufacturer's instructions.

Statistical Analysis

In the original analysis, we calculated the sample size on the hypothesis that simvastatin could reduce the time to reach clinical stability by 1.5 days. With a reference time to reach clinical stability of five days, we calculated that 175 patients were needed in each group to detect this difference with a power of 80% and a type 1 error of 5% (two-sided).

Categorical variables were described using counts and percentages. Continuous variables were expressed as the median and interquartile range. Baseline data between the two study groups were compared by means of the non-parametric Mann-Whitney U test for continuous data, and by the chi square test for categorical data. For 2 × 2 tables in which cells contained fewer than five observations, Fisher's exact two-tailed test for categorical data was used. The Wilcoxon signed-rank test was used to compare two related measurements. In addition, analysis of covariance (ANCOVA) was performed to compare the cytokines values at 48 hour in the two groups, adjusting for baseline values.

Data for the end-point was analysed on intention-to-treat-analysis. A P value of <.05 was considered statistically significant. All reported P values are two-tailed. All statistical calculations were performed using the Statistical Package for the Social Sciences (Version SPSS 15.01s) for Windows (SPSS Inc, Chicago, IL. USA).

RESULTS

The screening for inclusion criteria was started in December 2009 and ended in June 2011 due to slow recruitment because of the exclusion criteria of the study. Most excluded patients were receiving statins, other drugs that are metabolized by the CYP3A4 enzyme system or antibiotic therapy prior to enrolment. Thirty-four patients were randomly assigned and included in an intention-to-treat analysis for the end-point (19 to the simvastatin group and 15 to the placebo group). Figure 1 shows the study profile. The baseline characteristics of the patients at the time of enrolment were similar in the two groups and are detailed in Table 1. No significant differences between groups were documented in the clinical features and severity of patients, the aetiology of CAP, and the type of empirical antibiotic therapy and the time since hospital admission to antibiotic administration.

No significant differences in the time from hospital admission to clinical stability was found between study groups (median 3 days, IQR 2-5 vs 3 days, IQR 2-5; P=.47).

Table 2 compares serum cytokine concentrations and PaO2/FiO2 in the two groups. No significant differences in TNF-alpha, IL-6 and IL-10, C-reactive protein and PaO2/FiO2 levels at admission and at 48 hours of hospitalisation were found between simvastatin and placebo. However, there were significant changes in cytokine levels at admission compared with those at 48 hours during hospitalisation in each group (Figure 2). The median change from baseline to 48 hours of PaO2/FiO2 and cytokines between study groups did not differ significantly (Table 3). A *post hoc* subgroup analysis in patients with and without corticosteroids did not found significant differences among cytokines in the study groups (data not shown).

Transaminase (ALT and AST) and total creatine-kinase levels were similar in the simvastatin and placebo groups at 48 hours of hospitalisation (Table 4). One patient (in the simvastatin group) required ICU admission and one patient died (in the placebo group).

DISCUSSION

This is a prospective, randomized, double-blind, placebo-controlled trial that evaluated the use of statins in patients with CAP. We were unable to find that the use of 20 of simvastatin, once daily for four days in addition to the usual care, reduce the time from hospital admission to clinical stability and the concentrations of inflammatory cytokines in hospitalised patients with CAP.

No prior randomised study has evaluated the effect of statins on inflammatory cytokine levels or clinically relevant outcome parameters in hospitalised patients with CAP. Observational studies including prior users of the drug have related statin therapy with better outcomes in patients with CAP.[10-12] However, an observational study[14] suggested that the healthy user bias has a significant role as a confounding factor in the results. Certainly, the limitations of studies of this kind do not allow the application of their findings in clinical practice.

In a recent study involving adult intensive care patients with different infections and severe sepsis (mainly lung, urinary and intraabdominal infections), the investigators did not find differences in IL-6 concentrations between atorvastatin (20 mg daily) and placebo groups.[15] Importantly, another study did not support a beneficial effect of continuing pre-existing statin therapy (atorvastatin 20 mg daily) on sepsis and inflammatory parameters in patients with presumed infection;[16] no significant differences in IL-6 and C-reactive protein decreases were documented at any follow-up time-point in either study group. However, a randomised study in patients with acute bacterial infections found that statin therapy (40 mg of simvastatin, followed by 20 mg of simvastatin) was associated with a reduction in the levels of inflammatory cytokines.[17] A *post hoc* analysis of the subgroup of 48 patients with pneumonia revealed a significant decrease in IL-6 levels, but not in TNF-alpha levels. Nevertheless, it should be noted

that IL-6 levels increased at 72 hours in the placebo group. Moreover, a randomised trial found that the acute administration of 40 mg of atorvastatin daily in patients with sepsis may prevent sepsis progression.[18] The authors postulated that statins may modulate the pathophysiology of sepsis thereby restoring endothelial integrity and thus blocking one of the mechanisms in the development of multiorgan failure. Notably, inflammatory cytokines were not evaluated. Finally, a recent study documented that rosuvastatin did not improve clinical outcomes in patients with sepsis-associated acute respiratory distress syndrome.[19]

Our study suggests that simvastatin does not exert an effect on inflammatory cytokine levels in hospitalised patients with CAP. We evaluated the change in cytokine concentrations within the patient and between simvastatin and placebo groups. The cytokine concentrations decreased rapidly during the first days of hospital admission in both study groups, and no significant differences were documented at 48 hours in cytokine levels between simvastatin and placebo groups. However, cytokine levels were quantified only at baseline and at 48 h, which limits the assessment of statin effects on the further course of the inflammatory response. In addition, it is possible that higher doses of simvastatin[17] or the use of other statins[18] could have produced different results. A 20-mg dosage was selected to address concerns regarding potential toxicity. Interestingly, in a cecal ligation and perforation model of sepsis in mice, Merx *et al*. documented that anti-inflammatory properties vary between individual statins.[20]

Among the strengths of this study is the fact that the treatment was assigned on a random basis. Another unique feature of this trial was that it addressed the question of *de novo* statin use only in hospitalised patients with CAP. In this regard, a recent study documented major differences in the early status of the immune system in relation to the underlying type of infection

and concluded that therapeutic immunointerventions may be directed by the nature of infection.[21] In addition, importantly, our study suggest the safety profile of simvastatin in this context. However, it should be noted that patients receiving certain drugs that are metabolized by the CYP3A4 enzyme system were excluded. This because of the increased risk of rhabdomyolysis during concomitant use of simvastatin, a CYP3A4 substrate statin, and a CYP3A4 inhibitors[22] and legal aspects related to the responsibility insurance of the study.

Moreover, there are certain limitations that should be acknowledged. Firstly, the trial did not achieve its recruitment target for determining the effects of statins on time to reach clinical stability. Nevertheless, previous studies faced similar recruitment problems when conducting sepsis-related searches. Secondly, only systemic cytokine measurements were performed in our study, and this response might differ from that encountered in the lung. Similarly, biomarkers for evaluating coagulation or cardiovascular dysfunction were not evaluated. In addition, other potential benefits of statins in CAP were not assessed in the present study, including stabilization of cardiovascular system to avoid acute cardiac events and their potential antiviral and antibacterial effects. Thirdly, some baseline characteristics, such as corticosteroid use and long time to first antibiotic dose may complicate analysis of cytokine levels. However, no significant differences were observed between study groups regarding these topics. Finally, although our study population is representative of patients hospitalised with CAP since the clinical features were similar to those reported in other studies, the exclusion criteria are important limitations to the external validity of the results. In addition, a clinical trial designed with the exclusion criteria used in the present study is likely difficult, mainly due to recruit adequate number of patients.

In summary, we did not find that adding simvastatin, at a dose of 20 mg daily for four days, to the usual treatment of hospitalised patients with CAP decrease the time from hospital admission to clinical stability and the inflammatory cytokine levels. Due to the difficulty of recruiting patients without exclusion criteria, multicentre randomized studies are needed to determine the precise role of statins on clinically relevant outcome parameters in patients with this infection.

Competing interests: All authors have no conflicts of interest to disclose.

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Contributors: JC designed the study. DV, CGV, AFS, JD, and FL conducted the patient inclusion, reviewed all cases, collected patient information and compiled the data files. MM and FMR collected, processed and compiled the laboratory data. DV, CGV and AFS performed the statistical analyses. JC, DV and AFS drafted the paper. JD, MM, and FMR contributed to critical revision for important intellectual content. All authors approved the final manuscript. JC and DV are the guarantors.

Ethics approval: The study was approved by the Hospital Research Ethics Committee (AC099/08) and The Spanish Agency of Medicines and Medical Devices.

Data sharing statement: No additional data are available.

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Table 1 - Baseline characteristics according to study groups

	Simvastatin	Placebo
	n=19	n=15
Demographic data		
Age, median (IQR), years	63 (44.5-79)	76 (45.5-78)
Male sex	14 (73.7)	12 (80)
Current smoker	4 (21.1)	4 (26.7)
Comorbidities*	12 (63.2)	9 (60)
Charlson comorbidities index	1 (0-1.5)	1 (0-1)
Clinical features		
Impaired consciousness	2 (10.5)	2 (13.3)
Hypotension	1 (5.3)	2 (13.3)
Hypoxemia	12 (63.2)	9 (60)
Multilobar pneumonia	6 (31.6)	5 (33.3)
Leucocytosis (leukocytes >12 109/L)	14 (73.7)	8 (53.3)

IDSA / ATS criteria for ICU Admission[1]	4 (21%)	5 (33.3%)
CAP-specific scores		
High-risk PSI classes	8 (42.1)	8 (53.3)
Aetiology†		
All	11 (57.9)	11 (73.3)
S. pneumoniae	8 (42.1)	8 (53.3)
H. influenzae	0 (0)	2 (13.3)
Influenza A (H1N1)pdm09	1 (5.3)	1 (6.7)
Time to antibiotic administration, median (IQR), hours	5.5 (3-8)	5 (4-7.5)
Treatment at admission		
Corticosteroids	8 (42.1)	4 (26.7)
Beta-lactams	15 (78.9)	12 (80)
Quinolones	15 (78.9)	9 (60)
Mechanical ventilation	1 (5.3)	0 (0)

Data are reported as n (%), unless otherwise indicated. Abbreviations: IQR, interquartile range; PSI, pneumonia severity index.

*Comorbidities included chronic pulmonary diseases, chronic heart diseases, diabetes mellitus, chronic liver disease, chronic kidney disease, dementia and cerebrovascular disease.

†Other aetiologies in simvastatin group were *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* (one case each one).

Table 2 - Serum cytokine concentrations and PaO2/FiO2 upon enrolment and at 48 hours during hospitalization according to study groups

	Simvastatin n=19	Placebo n=15	P value
Within 24 h of admission			
PaO2/FiO2	276.1 (261-299)	276.3 (243-320)	.90
TNF-alpha (pg/ml)	24 (22.3-61.7)	30.6 (20.5-38)	.96
IL-6 (pg/ml)	700 (171-1908)	362 (239-515)	.91
IL-10 (pg/ml)	8.35 (1.5-38.8)	3.2 (2.4-8.3)	.17
At 48 h during hospitalization			
PaO2/FiO2*	300 (285-374)	338.1 (314-401)	.37
CRP (mg/dl)	151.2 (59.5-243.6)	69.4 (27.5-212.2)	.23
TNF-alpha (pg/ml)*	19.9 (16.7-40.8)	20.6 (15.8-25.5)	.58
IL-6 (pg/ml)*	141 (8-192)	66 (37.5-97)	.64
IL-10 (pg/ml)*	1.31 (0.4-3.8)	1.16 (0.45-2.2)	.61

* P values for ANCOVA analysis for PaO2/FiO2, TNF-alpha, IL-6 and IL-10 were 0.33,

0.97, 0.31 and 0.55 respectively. Data are reported as median (interquartile range). Abbreviations: CRP, C-reactive protein; IL, interleukin; PaO2/FiO2, partial pressure of arterial O2/fraction of inspired O2 ratio; TNF, tumor necrosis factor. CRP levels at baseline were not available.

Table 3 - Median change of serum cytokine concentrations and PaO2/FiO2 from baseline to 48 hours between study groups

	Simvastatin n=19	Placebo n=15	P value
PaO2/FiO2	-25.4 (0- -68.6)	-64.7 (15.5- -173.3)	.37
TNF-alpha (pg/ml)	5.1 (3.9-14.4)	10.2 (3.2-13.9)	.64
IL-6 (pg/ml)	463 (45.5-1579.5)	354 (169.5-413.5).87	.87
IL-10 (pg/ml)	4.1 (1.1-12.5)	1.8 (0.6-2.9)	.14

Data are reported as median (interquartile range). Abbreviations: IL, interleukin; PaO2/FiO2, partial pressure of arterial O2/fraction of inspired O2 ratio; TNF, tumor necrosis factor.

Table 4 – Adverse events during hospitalization according to study groups

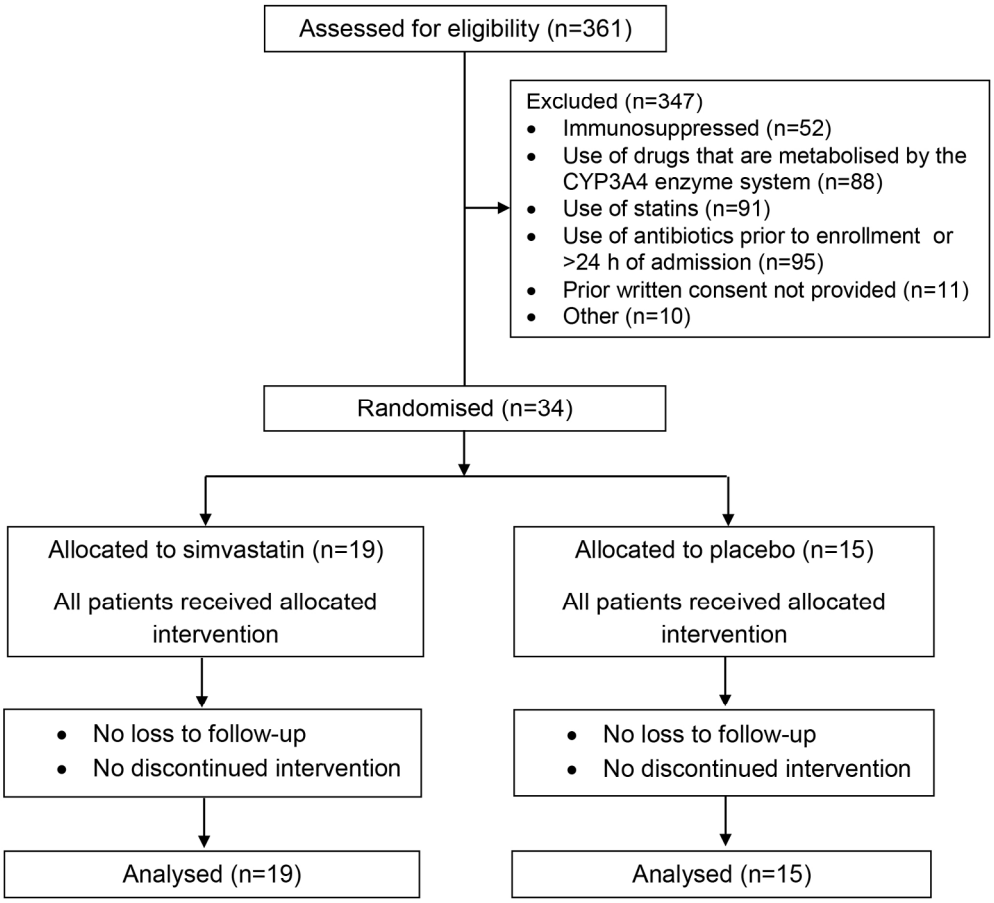
Adverse event	Simvastatin n=19	Placebo n=15	P value
AST level, median (IQR), (ukat/L)	0.25 (0.22-0.36)	0.7 (0.3-0.94)	.08
AST > 2 times upper reference limit	1 (5.2%)	3 (20%)	.30
ALT level, median (IQR), (ukat/L)	0.28 (0.22-0.43)	0.68 (0.3-1.0)	.19
ALT > 2 times upper reference limit	2 (10.5%)	2 (13.3%)	1
CK level, median (IQR), (ukat/L)	0.87 (0.51-2.22)	0.60 (0.32-2.7)	.53
CK > 2 times upper reference limit	1 (5.2%)	1 (6.6%)	1

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; IQR, interquartile range.

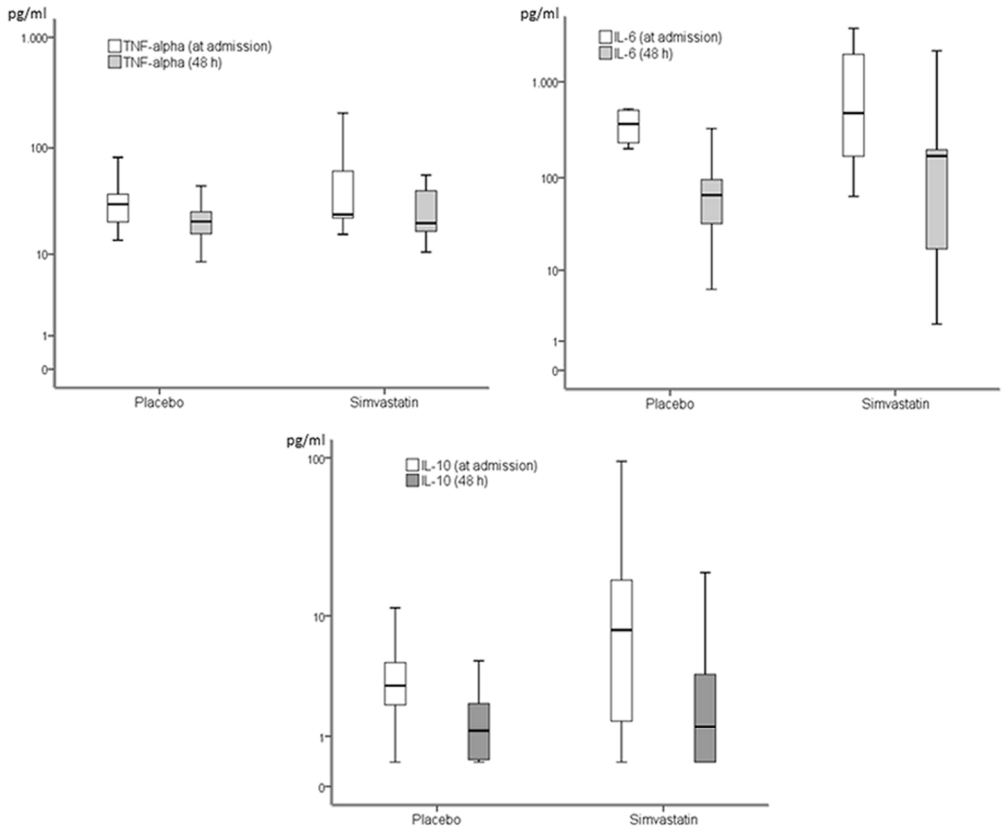
FIGURE 1. Flowchart of the trial

FIGURE 2. Changes in the cytokine concentrations at admission compared with those at 48 hours during hospitalization in each study group

Concentrations are showed in a logarithmic scale (y axis). All cases, Wilcoxon signed-rank test P<.001.



173x157mm (300 x 300 DPI)



133x111mm (300 x 300 DPI)

Items to include when reporting a randomized trial in a journal or conference abstract

Item	Description	Reported on line number
Title	Identification of the study as randomized	Title,4
Authors *	Contact details for the corresponding author	
Trial design	Description of the trial design (e.g. parallel, cluster, non-inferiority)	4
Methods		
Participants	Eligibility criteria for participants and the settings where the data were collected	7
Interventions	Interventions intended for each group	10,11
Objective	Specific objective or hypothesis	4,5
Outcome	Clearly defined primary outcome for this report	12-14
Randomization	How participants were allocated to interventions	10,11
Blinding (masking)	Whether or not participants, care givers, and those assessing the outcomes were blinded to group assignment	10,11
Results		
Numbers randomized	Number of participants randomized to each group	10
Recruitment	Trial status	
Numbers analysed	Number of participants analysed in each group	10
Outcome	For the primary outcome, a result for each group and the estimated effect size and its precision	19-21
Harms	Important adverse events or side effects	Page 3, lines 2-4
Conclusions	General interpretation of the results	Page 4, lines 5-7
Trial registration	Registration number and name of trial register	Page 4, line 9
Funding	Source of funding	Page 16

**this item is specific to conference abstracts*

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	3
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	6
	2b	Specific objectives or hypotheses	6,7
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	8,9
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	8
Participants	4a	Eligibility criteria for participants	8
	4b	Settings and locations where the data were collected	8
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	9
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	9
	6b	Any changes to trial outcomes after the trial commenced, with reasons	6,9
Sample size	7a	How sample size was determined	
	7b	When applicable, explanation of any interim analyses and stopping guidelines	
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	9
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	9
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	9
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	9
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	9

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	11
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	11
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	12, Figure 1
	13b	For each group, losses and exclusions after randomisation, together with reasons	Figure 1
Recruitment	14a	Dates defining the periods of recruitment and follow-up	8
	14b	Why the trial ended or was stopped	12
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	12, Figure 1
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	12
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	13, Table 4
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	15,16
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	16
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	16,17
Other information			
Registration	23	Registration number and name of trial registry	4
Protocol	24	Where the full trial protocol can be accessed, if available	
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	18

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.