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ORIGINAL ARTICLE

Corticosteroid use, myocardial injury and in-hospital cardiovascular events in patients with community-acquired pneumonia

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Background and Purpose: Corticosteroids are often prescribed to community-acquired pneumonia (CAP) patients, but the relationship with major cardiovascular events (MACEs) is unclear.

Experimental Approach: 541 CAP patients were recruited (334 males, mean age 71.9 ± 16.2 years). High-sensitivity troponin T (hs-cTnT) was measured at admission, during the hospital stay and at discharge. MACE occurrence was registered during a long-term follow-up.

Key Results: Overall, 318 patients (59%) showed hs-cTnT elevation >99th percentile (>0.014 μg/L). Age, heart failure and the increasing quintiles of hs-cTnT (hazard ratio [HR] 2.16, 95% confidence interval [CI] 1.82-2.58, $P < .001$) predicted MACEs. Among patients with hs-cTnT >0.014 μg/L at admission, 102 patients (31%) were on corticosteroids and showed lower hs-cTnT increase ($P = .021$), (NADPH) oxidase-2 (Nox2) activation ($P = .005$) and incidence of MACEs than untreated ones (HR 0.64, 95% CI 0.41-0.97, $P = .038$); no effect of corticosteroids on MACEs was observed in CAP patients with normal troponin. In vitro study showed that glucocorticoids have an antioxidant effect via downregulation of Nox2 activity.

Conclusion and Implications: The study provides evidence that corticosteroid use is associated with lower increase of hs-cTnT and incidence of MACEs in CAP patients.

KEYWORDS
glucocorticoids, myocardial injury, pneumonia, troponins

Roberto Cangemi and Roberto Carnevale, contributed equally to this work

The authors confirm that the Principal Investigator for this paper is Francesco Violi and that he had direct clinical responsibility for patients.

1 | INTRODUCTION

Despite improved management by antibiotic therapy the risks of hospitalization, morbidity and mortality are still elevated in community-acquired pneumonia (CAP) patients.[1] Cardiovascular disease represents a harmful complication occurring in the early phase of hospitalization.[2,3] In a large prospective study including 1182 CAP patients, cardiovascular events such as myocardial infarction (MI), heart failure and stroke occurred in 32% of patients during the first 48 hours from admission and increased the risk of mortality and cardiovascular recurrences in short- and long-term follow-up.[4]

In accordance with this finding, we have previously reported that CAP patients display an early increase of cardiac troponin, in >50% of patients, which was accompanied to electrocardiogram (ECG) modification compatible with non-ST-elevation myocardial infarction (NSTEMI) in the majority of cases[5].

The impact of corticosteroid use in CAP patients provided conflicting results with meta-analyses showing a positive effects in terms of reduction of death,[6,7] an effect, however, not confirmed by others.[8,9] Accordingly, guidelines form American Thoracic Society and the Infectious Diseases Society of America advise against the use of corticosteroids in CAP unless there are precise indications for their use, as in case of coexistent asthma, chronic obstructive pulmonary disease (COPD) or autoimmune diseases.[10]

Corticosteroids seem to also have an effect on cardiac complications of CAP patients, as shown by a retrospective study conducted in 493 CAP patients, in which we found that corticosteroid users presented a significant reduction of MI compared to the nonusers.[11] However, this beneficial effect was limited to patients with concomitant COPD.

Due to the negative association between myocardial injury and long-term adverse outcomes, we speculated that corticosteroids may prevent troponin release and eventually reduce major adverse cardiovascular events (MACEs).

Previous studies reported a relationship between oxidative stress as assessed by blood levels of soluble (NADPH) oxidase-2 (Nox2)-derived peptide (*sNox2.dp*),[11–13] a marker of Nox2, and troponin increase in CAP.[14] Based on this, we explored if corticosteroid treatment may reduce the blood levels of sNox2-dp and the relationship with troponin values. Furthermore, we performed an in vitro study to investigate if corticosteroids, at the same concentration detectable in corticosteroid-treated patients, possess an antioxidant effect via regulation of Nox2 activity.

What is already known about this subject

- Patients with community-acquired pneumonia may suffer from myocardial injury, which is associated with increased risk of major adverse cardiovascular events.
- Corticosteroids can be prescribed to these patients, but the relationship between their use, myocardial injury and outcomes in these patients is unknown.

What this study adds

- The present study provides evidence that corticosteroid treatment may protect against myocardial injury and poor cardiovascular outcomes in patients hospitalized for community-acquired pneumonia.

2 | MATERIALS AND METHODS

2.1 | Patients

We analysed data from a prospective observational study aimed to evaluate the incidence of major vascular events in hospitalized adult patients with pneumonia (clinical.trial.gov: NCT01773863).

This cohort study prospectively recruited and followed up patients referred to three medical centres from the University-Hospital Policlinico Umberto I, Sapienza University of Rome, Italy: the Department of Internal Medicine and Medical Specialties, the Department of Clinical Medicine and the Department of Public Health and Infectious Diseases.

We enrolled adults who met the following criteria: (a) age ≥18 years; (b) clinical presentation of an acute illness with at least two or more of the signs or symptoms of CAP, as previously reported; and (c) presence of new consolidation(s) on chest X-ray.[15] Pneumonia was defined as CAP diagnosed on hospitalization in a patient who did not meet the criteria for healthcare-associated pneumonia.[16]

Exclusion criteria were immunosuppression (HIV infection, chemotherapy, high dose of immunosuppressive agents to prevent the rejection of transplanted organs and tissues or to treat autoimmune diseases), critical illness requiring admission to an intensive care unit,[17] presence of malignancy, pregnancy or breastfeeding, documented severe allergy to antibiotics and healthcare-associated pneumonia.[16]

All patients with CAP admitted to the three units from October 2011 to January 2018, gave written informed consent and were prospectively recruited and followed up. The present study was conducted according to the principles stated in the Declaration of Helsinki. The study was approved by the local Ethics Committee (Prot. n. 864/11).

2.1.1 | Baseline assessment

Data regarding demographic characteristics, comorbidities and concurrent therapy were collected after inclusion in the study. The severity of CAP was estimated by the Pneumonia Severity Index (PSI), a

validated prediction score for 30-day mortality in patients with CAP.[18] For each patient recruited, 12-lead ECG and routine blood laboratory tests were immediately performed after admission. High-sensitivity troponin (hs-cTnT) was measured at baseline and every 24 hours up to 3 days from admission and at hospital dismission.

Pre-existence of type 2 diabetes mellitus (T2DM), hypertension, coronary heart disease (CHD), dyslipidaemia, peripheral artery disease (PAD), COPD, heart failure (HF), chronic (persistent or permanent) atrial fibrillation (CAF) and paroxysmal atrial fibrillation (PAF) were defined as previously described.[19]

In-hospital systemic corticosteroid therapy was determined by the clinical judgment of the treating physician. Patients were considered as treated with systemic corticosteroids if the treatment was initiated withing 1 day from hospital admission.

2.1.2 | In-hospital outcomes

The occurrence of a MI was diagnosed according to the Third Universal Definition of Myocardial Infarction.[20] ST-elevation MI (STEMI) and NSTEMI were defined as previously reported,[20] and were confirmed by cardiologists.

Cardiovascular death included fatal MI, fatal stroke, sudden death, death due to cardiogenic shock in patients with HF, pulmonary embolism, rupture or dissection of aneurysm, death related to cardiovascular investigation/procedure/operation, and death due to other specified cardiovascular causes.

In-hospital death for noncardiovascular causes was considered a censoring event.

2.1.3 | Long-term follow-up

Follow-up data about MACEs (MI and cardiovascular death) were obtained by review of hospital databases, medical records, death certificates or telephone interviews. Adjudication of the events was performed by a central adjudication committee (CC and SM) who did not participate in the patients' recruitment and follow-up and were unaware of the clinical and laboratory characteristics of any patient.

Censored cases included patients who refused to participate in the follow-up interviews after hospital dismission, patients who stopped answering telephone calls or refused telephone interviews during the follow-up and patients who died from noncardiovascular causes.

2.2 | Isolation of human peripheral blood mononuclear cells (PBMCs)

Freshly taken EthylenDiaminoTetracetyc Acid (EDTA)-blood from healthy subjects (HS) was diluted with phosphate buffer saline (PBS) (1:4), stratified over 10 mL of Ficoll-Paque and then centrifuged at 1006 g for 30 minutes at 20 °C. The mononuclear cell layer was aspired and transferred into a 50-mL conical tube and washed twice with PBS by centrifugation at 1000*g* for 10 minutes. The cell pellet was suspended in 1 mL of PBS and was pre-incubated (20 minutes at 37 °C) with a scalar concentration (150-300-600 ng/mL) of methylprednisolone (Sigma-Aldrich, St. Louis, Missouri, USA) or betamethasone (Sigma Aldrich), which corresponded to the levels detected in the serum of patients on glucocorticoid administration.[21] After incubation, samples were stimulated with lipopolysaccharide (LPS)[22] (40 pg/mL; Sigma Aldrich) for 10 minutes at 37 °C. Cells were centrifuged for 3 minutes at 1006 g. Supernatants and pellet were stored at −80 °C for analysis of sNox2dp, H_2O_2, $p47^{phox}$ and pAKT.

2.3 | Serum and platelet sNox2-dp

Soluble Nox2-derived peptide (sNox2-dp) was measured with an ELISA method as previously reported.[23] Values were expressed as pg/mL and the intra-assay and inter-assay coefficients of variation were 8.95% and 9.01%, respectively.

2.4 | H_2O_2 determination

Hydrogen peroxide (H_2O_2)[24–27] was evaluated by a Colorimetric Detection Kit (Arbor Assays, Ann Arbor, Michigan, USA) and expressed as μM. The intra-assay and inter-assay coefficients of variation were 2.1% and 3.7%, respectively.

2.5 | Western blot analysis of AKT and $p47^{phox}$ phosphorylation

The phosphorylation of AKT and $p47^{phox}$ was analysed in cell pellets from HS. Cells were suspended in a 2 × lysis buffer (5 mM EDTA, 0.15 mol NaCl, 0.1 mol TRIS, pH 8.0, 1% triton and 10 μg/mL of protease and phosphatase inhibitors cocktail; Thermo Fisher Scientific, Waltham, Massachusetts, USA). Equal amounts of protein (30 μg/lane) were solubilized in a 2X Leammli sample buffer, separated on 10-12% SDS-polyacrylamide gel and then electro-transferred to nitrocellulose membranes. After blocking with Bovine Serum Albumin (BSA, 5%; Sigma-Aldrich, St. Louis, Missouri, USA) the membranes were incubated overnight at 4 °C with rabbit polyclonal anti-p-p47phox (Abcam, Cambridge, UK), anti-p-AKT and anti-β-Actin antibody (Santa Cruz Biotechnology, Inc., Dallas, Texas, USA), and subsequently with secondary antibody (1:3000; Bio-Rad, Hercules, California, USA). The immune complexes were detected by enhanced chemiluminescence substrate (ECL Substrates, Bio-Rad). Densitometric analysis of the bands was performed using Image J software.

2.6 | Serum hs-cTnT measurement

hs-cTnT levels were measured using an Elecsys 2010 (Roche Diagnostics, Indianapolis, IN, USA) at a dedicated core laboratory. According to the manufacturer, the 99th-percentile cut-off point for hs-cTnT is 0.014 μg/L, and a coefficient of variation of <10% is achieved at 0.013 μg/L.[28]

2.7 | Statistical analysis

Categorical variables are reported as counts and percentages, and continuous variables as mean ±SD, or medians and interquartile ranges (IQRs). Differences between percentages were assessed by chi-square or Fisher exact tests. Student unpaired *t*-tests and analysis of variance were used for normally distributed continuous variables. Appropriate nonparametric tests (Mann-Whitney, Kruskal-Wallis and Spearman rank correlation tests) were used for all other variables.

Data were analysed for the assessment of treatment effect on biomarkers performing a repeated measures MANOVA with one between-subject factor (treatment group) and one within-subject factor (time at two or three levels). As covariates, we considered the possible random differences in age and comorbidities between the groups.

After dividing the population into groups, the cumulative incidence was estimated using a Kaplan-Meier product-limit estimator. Survival curves were formally compared using the log-rank test. Cox proportional hazards analysis was used to calculate the adjusted relative hazards of outcome events by each clinical variable.

For multivariate models, model selection was performed using forward stepwise regression on the basis of the Akaike information criterion.

Only *P* values <.05 were considered statistically significant. All tests were two-tailed, and analyses were performed using computer software packages (R version 2.15.2, R Development Core Team, Wien, Austria).

2.8 | Nomenclature of Targets and Ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY, and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.[29]

3 | RESULTS

A total of 541 participants were recruited (334 men, mean age 71.9 ± 16.2 years).

During the in-hospital stay, 318 patients (59%) disclosed hs-cTnT elevation above the 99th percentile (>0.014 μg/L).

Patients with hs-cTnT elevation were older, more likely to be former smokers and had a higher prevalence of history of comorbidities like hypertension, CHD, stroke, diabetes, heart failure and AF compared to patients with normal hs-cTnT. Moreover, they were more likely to be treated with aspirin,[30–32] thienopyridines, anticoagulants and statins, and belong to higher severity PSI classes than patients with normal hs-cTnT (Table 1).

Patients with hs-cTnT elevation at admission showed a progressive increase of hs-cTnT during the in-hospital stay that decreased at hospital dismission: from 0.032 (0.021-0.061) μg/L to a maximum of 0.047 (0.026-0.098) μg/L (*P* < .001) reached 24-72 hours from hospital admission to 0.030 (0.016-0.054) μg/L (*P* < .001) at hospital dismission.

On the contrary, patients without hs-cTnT elevation did not show any significant change in hs-cTnT level during the in-hospital stay (from 0.010 [0.08-0.011] to 0.010 [0.08-0.012] μg/L at 24-72 hours from hospital admission, *P* = .316).

3.1 | Troponins and MACE at long-term follow-up

Patients were followed for a median time of 22.7 months (IQR 7.2-43.2 months), yielding a total of 1175 patient-years of observation

During the entire follow-up, 140 patients experienced a MACE (66 nonfatal MI, 74 cardiovascular deaths).

In the whole cohort, a multivariable COX regression analysis showed that age (hazard ratio [HR] 1.06, 95% confidence interval [CI] 1.04-1.08, *P* < .001), in-hospital maximum levels of hs-cTnT (HR 2.02, 95% CI 1.59-2.58, *P* < .001), heart failure (HR 1.90, 95% CI 1.23-2.95, *P* = .004), and CHD (HR 1.65, 95% CI 1.09-2.49, *P* = .018) independently predicted MACEs after adjusting for sex, smoking habit, COPD, arterial hypertension, CKD, diabetes, history of stroke, PAF and CAF.

To better stratify MACE risk in relation to troponin levels, in-hospital maximum hs-cTnT was stratified in five quintiles (≤0.010, 0.011-0.014, 0.015-0.030, 0.031-0.074 and >0.074 μg/L). A multivariable COX regression analysis showed age, heart failure, CHD and the increasing quintiles of hs-cTnT (HR 2.16, 95% CI 1.82-2.58, *P* < .001) predicted MACEs after adjusting for the possible confounding variable (Figure 1).

3.2 | Systemic corticosteroids and troponins

In the whole cohort, 137 patients (25%) were treated with systemic corticosteroids started at hospital admission. Corticosteroid treatment encompassed **methylprednisolone**[33] (60%, 20-80 mg/d), **betamethasone** (22%, 4-8 mg/d) and **prednisone** (18%, 25-50 mg/d).

Corticosteroid-treated patients were older and more likely to be former smokers; moreover, they showed a higher prevalence of COPD and AF, and belonged to higher severity PSI classes than non-treated patients (Table 2).

TABLE 1 Clinical characteristics of patients with or without troponin elevation

	Total patients	Patients with hs-cTnT ≤ 0.014 μg/L	Patients with hs-cTnT > 0.014 μg/L	*P* value
n	541	223	318	
Age (years)[a]	71.9 ± 16.2	62.2 ± 17.7	78.4 ± 11.1	< 0.001
Male sex (%)	62	60	64	0.406
Smokers (%)	22	26	19	0.064
Former smokers (%)	34	28	38	0.012
Arterial hypertension (%)	72	59	80	< 0.001
Coronary heart disease (%)	30	15	40	< 0.001
Heart failure (%)	22	6	32	< 0.001
History of stroke (%)	12	6	15	0.002
Diabetes (%)	26	14	34	< 0.001
COPD (%)	32	23	38	< 0.001
Paroxysmal AF	15	7	22	< 0.001
Persistent/permanent AF	13	7	18	< 0.001
PSI class II (%)	26	50	10	< 0.001
PSI class III (%)	24	29	21	
PSI class IV (%)	37	20	47	
PSI class V (%)	13	1	22	
Aspirin (%)	32	23	38	< 0.001
Thienopyridines (%)	13	14	18	< 0.001
Heparins (%)	6	2	9	0.001
OAC (%)	14	10	17	0.035
Statins (%)	30	23	34	0.005

Abbreviations: AF, atrial fibrillation; COPD, chronic obstructive pulmonary disease; OAC, oral anticoagulants; PSI, pneumonia severity index.
[a]Data are expressed as mean ± standard deviation.

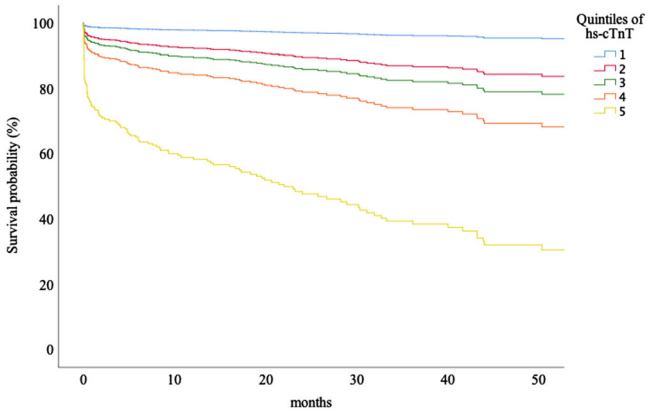

FIGURE 1 Cumulative MACE survival probability at long-term follow-up, according to the quintiles of maximum levels of hs-cTnT during the in-hospital stay. HR 2.16, 95% CI 1.82-2.58, *P* < .001 for each increasing quintile (multivariable COX regression analysis)

Among patients with hs-cTnT > 0.014 μg/L (n = 318), 102 patients (31%) were treated with systemic corticosteroids. In this group, corticosteroids-treated patients were older and showed a tendency to a higher prevalence of COPD compared to nontreated patients (Table 3).

Among patients with elevated troponins, no baseline differences were found between patients treated or not with corticosteroids (0.033 [0.23-0.064] *vs* 0.031 [0.021-0.059] μg/L, *P* = .646). In both groups, hs-cTnT levels significantly increased between 24 and 72 hours from hospital admission (0.042 [0.025-0.082] μg/L, *P* < .001 and 0.050 [0.027-0.108] μg/L, *P* < .001, respectively), but the increase in hs-cTnT was significantly lower in the corticosteroid-treated patients compared to untreated patients (0.006 [0.00-0.019] *vs* 0.012 [0.002-0.040] μg/L, *P* = .003). At discharge, both groups showed similar hs-cTnT levels. A MANOVA analysis confirmed an effect of corticosteroids on hs-cTnT after adjusting for age, coronary heart disease, heart failure and COPD (*F* = 4.1, *P* = .021) (Figure 2). Among patients with hs-cTnT ≤ 0.014 μg/L, no significant differences in hs-cTnT levels were found between patients treated or not with corticosteroids at baseline (0.010 [0.007-0.012] *vs* 0.010 [0.008-0.011] μg/L, *P* = .296] and at 24-72 hours from hospital admission (0.010 [0.008-0.012] *vs* 0.010 [0.009-0.011] μg/L, *P* = .403].

3.3 | Corticosteroids and MACEs at long-term follow-up

Among patients with hs-cTnT > 0.014 μg/L, corticosteroid-treated patients showed a lower incidence of MACEs than untreated ones [29% (29/99) *vs* 43% (92/213), *P* = .042] during the follow-up (Figure 3).

TABLE 2 Clinical characteristics of patients treated or not with systemic corticosteroids

	Total patients	Not corticosteroid treated	Corticosteroid treated	*P* value
n	541	403	138	
Age (years)[a]	71.9 ± 16.2	69.9 ± 17	77.5 ± 11.5	< 0.001
Male sex (%)	62	62	63	0.773
Former smokers (%)	31	41	34	0.042
Arterial hypertension (%)	72	71	75	0.345
Coronary heart disease (%)	30	28	33	0.290
Heart failure (%)	22	21	24	0.525
History of stroke (%)	12	12	13	0.646
Diabetes (%)	26	24	32	0.074
COPD (%)	32	27	47	< 0.001
Paroxysmal AF	15	13	21	0.055
Persistent/permanent AF	14	11	21	0.004
PSI class II (%)	26	30	14	< 0.001
PSI class III (%)	24	25	22	
PSI class IV (%)	37	34	45	
PSI class V (%)	13	11	19	
Aspirin (%)	32	33	32	0.861
Thienopyridines (%)	13	12	14	0.628
Heparins (%)	6	5	8	0.228
OAC (%)	14	13	18	0.120
Statins (%)	30	30	30	0.949

Abbreviations: AF, atrial fibrillation; COPD, chronic obstructive pulmonary disease; OAC, oral anticoagulants; PSI, pneumonia severity index.
[a]Data are expressed as mean ± standard deviation.

A COX regression analysis confirmed that in-hospital corticosteroid use was inversely associated with MACE (HR 0.64, 95% CI 0.41-0.97, $P = .038$), while age (HR 1.05, 95% CI 1.03-1.08, $P < .001$), CHD (HR 1.6, 95% CI 1.05-2.33, $P = .029$) and congestive heart failure (HR 1.72, 95% CI 1.15-2.58, $P = .009$) were positively associated to MACEs after adjusting for sex, smoking habit, COPD, arterial hypertension, CKD, diabetes, history of stroke, PAF and CAF.

Among patients with hs-cTnT > 0.014 μg/L, 112 patients died during follow-up (34% of corticosteroid-treated patients and 36% of not-treated patients, $P = .899$), 49 for noncardiovascular causes (17% of corticosteroid-treated patients and 15% of not-treated patients, $P = .616$).

Among patients with hs-cTnT ≤ 0.014 μg/L, the incidence of MACEs did not differ in patients treated or not with corticosteroids (14% *vs* 7%, $P = .222$).

3.4 | Corticosteroids and sNOX2-dp

At hospital admission, patients disclosed elevated levels of serum sNOX2-dp that correlated with baseline hs-cTnT (rho Spearman [Rs] 0.464, $P < .001$) and maximum levels of hs-cTnT (Rs 0.525, $P < .001$); sNox2 decreased at hospital discharge (27 [17-41] *vs* 22 [15-33] pg/mL, $P < .001$).

A MANOVA analysis showed that sNox2-dp decreased over time more in corticosteroid-treated patients compared to not-treated patients, also after adjusting for possible confounding factors (age, PSI classes, COPD, AF, smoking habit) ($F = 7.9$, $P = .005$) (Figure 4A).

After dividing the whole cohort into patients with or without troponin elevation, the effect of corticosteroid treatment over time on sNOX2-dp remained significant in patients with hs-cTnT > 0.014 μg/L ($F = 6.4$, $P = .012$) (Figure 4B) but not in patients with hs-cTnT ≤ 0.014 μg/L ($F = 1.3$, $P = .261$) (Figure 4C).

3.5 | In vitro study

3.5.1 | Glucocorticoids and LPS-induced oxidative stress

Betamethasone- and methylprednisolone-treated cells stimulated with LPS (40 pg/mL) showed a significant decrease in sNox2-dp compared to LPS-stimulated cells alone (Figure 5A). This effect was dose-dependent with a significance reached at concentrations of 300 and 600 ng/mL compared to 150 ng/mL (Figure 5A). Furthermore, both betamethasone- and methylprednisolone-treated cells stimulated with LPS encompassed a significant decrease of H_2O_2 production compared to LPS-stimulated cells alone (Figure 5B). This effect was dose-dependent with a significance reached at concentrations of 300 and 600 ng/mL compared to 150 ng/mL (Figure 5B).

TABLE 3 Clinical characteristics of patients with troponin elevation, according to corticosteroid treatment

	Non-corticosteroid group	Corticosteroid group	*P* value
n	216	102	
Age (years)[a]	77.6 ± 11.7	80.2 ± 9.4	0.044
Male sex (%)	64	64	0.977
Former smokers (%)	37	41	0.522
Arterial hypertension (%)	82	76	0.227
Coronary heart disease (%)	41	37	0.563
Heart failure (%)	34	27	0.214
History of stroke (%)	15	16	0.959
Diabetes (%)	34	35	0.884
COPD (%)	35	45	0.086
Paroxysmal AF	19	27	0.144
Chronic AF	17	21	0.456
PSI class II (%)	11	7	0.273
PSI class III (%)	23	17	
PSI class IV (%)	45	52	
PSI class V (%)	21	24	
Aspirin (%)	41	32	0.126
Thienopyridines (%)	18	17	0.729
Heparins (%)	9	9	0.960
OAC (%)	15	20	0.350
Statins (%)	36	31	0.415

Abbreviations: AF, atrial fibrillation; COPD, chronic obstructive pulmonary disease; OAC, oral anticoagulants; PSI, pneumonia severity index.
[a]Data are expressed as mean ± standard deviation.

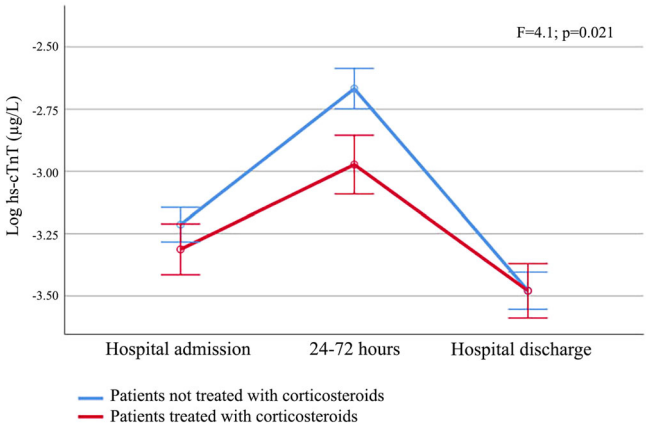

FIGURE 2 Troponin levels during hospitalization according to corticosteroid treatment in patients with hs-cTnT > 0.014 µg/L. Data are expressed as adjusted marginal means (±SE) of log-transformed hs-cTnT levels (MANOVA analysis)

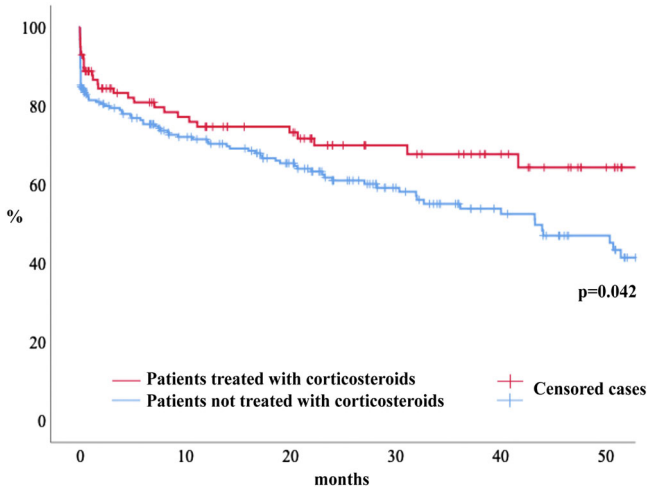

FIGURE 3 Kaplan-Meier estimates of time to MACEs according to in-hospital corticosteroid treatment among patients with hs-cTnT > 0.014 µg/L during the in-hospital stay (log-rank test)

3.5.2 | Intra-signalling pathway of Nox2 activation by LPS

To investigate upstream pathways implicated in Nox2 activation we analysed the role of AKT, which is implicated in $p47^{phox}$ activation and ultimately Nox2 upregulation.[34] Betamethasone- and methylprednisolone-treated cells stimulated with LPS showed a significant decrease of AKT phosphorylation compared to LPS-treated cells alone (Figures 6A,B). This effect was dose-dependent with a significance reached at concentrations of 300 and 600 ng/mL compared to

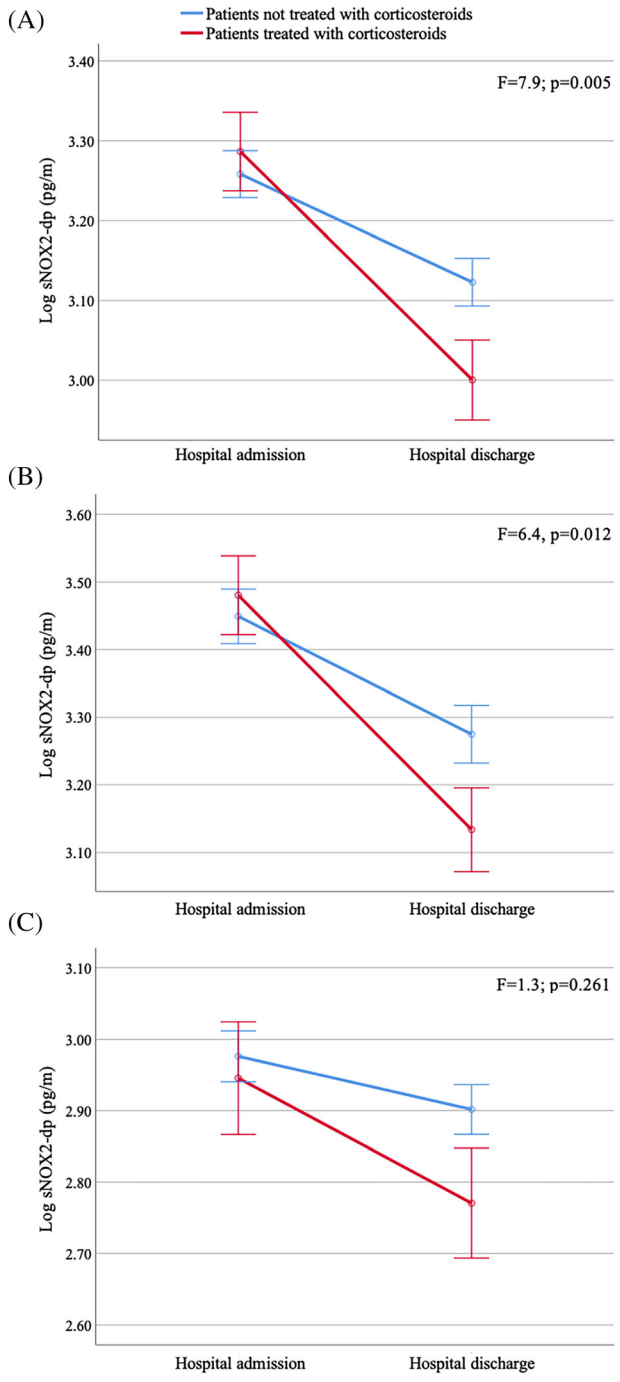

FIGURE 4 Serum sNox2-dp levels during hospitalization according to corticosteroid treatment in the whole cohort (panel A), in patients with hs-cTnT > 0.014 μg/L (panel B) and in patients with hs-cTnT ≤ 0.014 0.014 μg/L (panel C). Data are expressed as adjusted marginal means (±SE) of log-transformed sNox2-dp levels (MANOVA analyses)

150 ng/mL (Figure 6A,B). Similarly, betamethasone- and methylprednisolone-treated cells stimulated with LPS reduced $p47^{phox}$ phosphorylation compared to LPS-treated cells alone, an effect reached with 300 and 600 ng/mL of betamethasone and methylprednisolone (Figures 6C,D).

4 | DISCUSSION

The study provides evidence that glucocorticoids are associated with a reduction of myocardial injury in patients admitted with CAP. Furthermore, corticosteroids were associated with a lower incidence of MACEs in a long-term follow-up. The effect of corticosteroids seems to be mediated by an antioxidant mechanism related to Nox2 downregulation.

Previous studies showed that myocardial injury, as assessed by cardiac troponin elevation, is commonly detected in pneumonia. In a cohort of hospitalized CAP, we previously showed that elevated levels of hs-cTnT (>0.014 μg/L, ie, over the 99th percentile) were found in more than 50% of patients and associated with in-hospital MI in about 10% of patients.[5] The increase of hs-cTnT was mostly detected within 24-72 hours from hospital admission, indicating that myocardial damage, as well as the risk of MI, were maximally evident in the first days of hospitalization.

In a cohort of 295 CAP patients in the Netherlands, Vestjens et al confirmed that hs-cTnT elevation is common at hospital admission and showed that hs-cTnT elevation is an independent predictor of short- and long-term mortality.[35] Recently, in a cohort of 730 CAP patients followed up for 1 year, Menéndez found that hs-cTnT elevation at admission independently predicted early but not late cardiovascular events.[36] In the present study, we extended these previous reports, underlying the importance of measuring hs-cTnT to identify patients at risk for cardiovascular events. In our study, hs-cTnT were evaluated not only at admission, but also every 12 hours up to 72 hours. Thus, we showed that maximum levels of in-hospital hs-cTnT (ie, at 24-72 hours) were a strong predictor of MACEs in a dose-dependent fashion during long-term follow-up.

We recently reported a potential role for systemic corticosteroids in reducing MI risk in patients with CAP, indicating that this drug category could encompass a vascular protection effect in this setting.[21] However, no data exist about the potential effect of corticosteroid on in-hospital myocardial injury or long-term cardiovascular events. In the present study we found that systemic corticosteroids reduced troponin increase in patients with troponin elevation during hospitalization, while no changes were detected in patients with normal troponin. Moreover, in patients disclosing troponin increase, in-hospital corticosteroid use was independently associated with a reduction in MACEs incidence at long-term follow-up. Interestingly, no difference in MACE incidence was found in patients who did not disclose troponin increase during hospitalization.

Troponin elevation has been associated with Nox2 upregulation in patients with CAP, suggesting that Nox2- dependent oxidative stress is implicated in myocardial injury.[14] Nox2 is among the most important enzymes generating reactive oxidant species (ROS) in the vasculature. ROS production has been proved to have several detrimental effects on the myocardium, such as apoptotic cell death, hypertrophy and dysfunction.[37] Numerous studies have indicated that Nox2 localizes in cardiomyocytes, where it could play an important role in redox balance,[38,39] suggesting that enhanced Nox2-derived oxidative stress may be implicated in myocardial injury. Overactivation

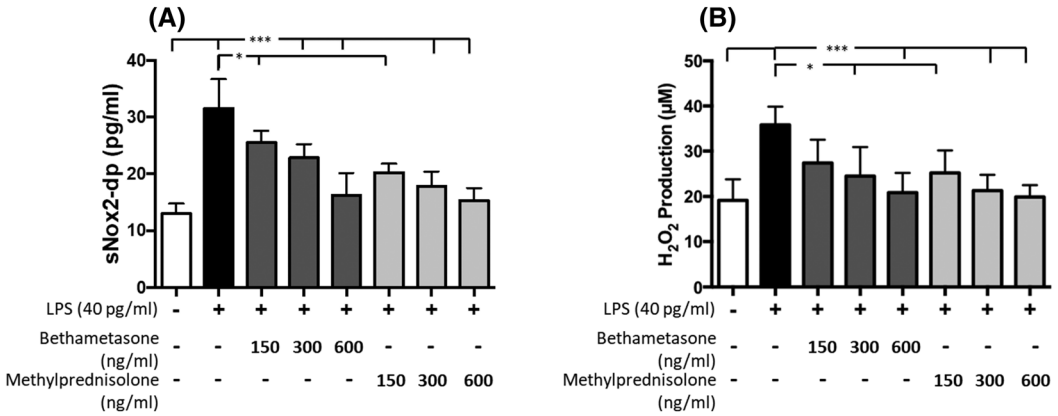

FIGURE 5 Glucocorticoids and oxidative stress. Nox2 activation (A) and H_2O_2 production (B) were evaluated in human peripheral blood mononuclear cells (PBMCs) incubated with scalar concentrations (150-600 ng/mL) of betamethasone or methylprednisolone and stimulated with LPS (40 pg/mL) (n = 5 experiments) (*P < .05, **P < .001;***P < .0001, Kruskal-Wallis test)

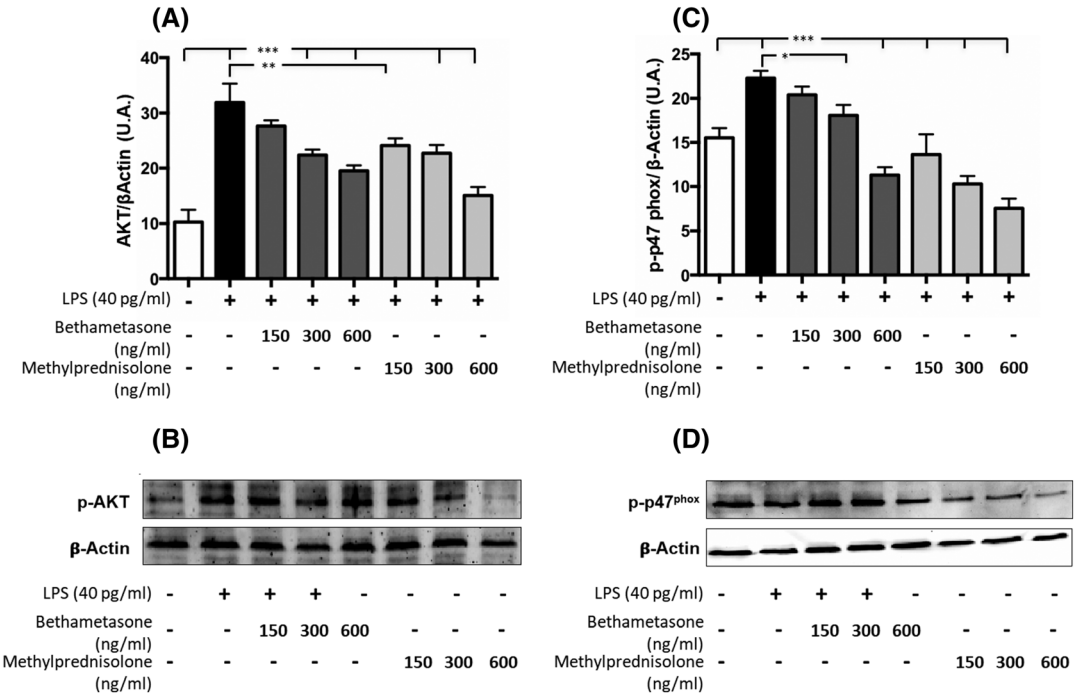

FIGURE 6 Glucocorticoids and pathway of oxidative stress. AKT (A) and p47phox (C) phosphorylation were analysed in human peripheral blood mononuclear cells (PBMCs) incubated with scalar concentrations (150-600 ng/mL) of betamethasone or methylprednisolone and stimulated with LPS (40 pg/mL) (n = 3 experiments) (*P < .05, **P < .001, ***P < .0001, Kruskal-Wallis test). A representative western blot of AKT (B) and p47phox (D) phosphorylation in the presence or not of betamethasone or methylprednisolone (150-600 ng/mL)

of Nox2 in CAP patients may be explained by previous reports indicating that single-stranded RNA viruses irrespective of their classification, including influenza A virus and DNA viruses, infect cells via Toll-like 7-mediated Nox2 activation and that the virus pathogenicity is abolished in Nox2 knockout cells.[40]

We investigated if corticosteroid use could reduce Nox2 activation in this setting. At admission, patients disclosed elevated concentrations of sNox2-dp, a marker of Nox2 activation, that correlated to hs-cTnT levels and were reduced at hospital dismission. Patients treated with systemic corticosteroid showed a stronger reduction in serum sNox2-dp than not-treated patients; such a phenomenon was particularly evident in patients disclosing troponin elevation. To explore the biological plausibility of this finding, we performed in vitro experiments to assess if corticosteroids modulate Nox2 activation and ROS production. These experiments showed that corticosteroids, at concentrations detected in human blood in after administration, decreased Nox2 activation and H_2O_2 production in LPS stimulated cells through a reduction in AKT and $p47^{phox}$ phosphorylation.

The present study has limitations and implications. The study supports and extends previous reports indicating that patients with

troponin elevation are at higher risk of long-term MACEs. Patients with troponin elevation may benefit from corticosteroid treatment to improve vascular outcomes. However, the results of our study could be biased by its retrospective nature and by the fact that patients were not randomized to corticosteroid treatment, which was given at the discretion of the managing physician for perceived need. Moreover, the relatively small sample size of our study did not allow us to evaluate the potential different effect of each corticosteroid. However, corticosteroid-treated patients were older and with more comorbidities, such as COPD, thus this fact should reinforce the results of the present report. Even if the antioxidant effect reported here and previous reports showing glucocorticoids' antiplatelet effects[21] could explain the cardiovascular protection, the reasons for MACE reduction during the long-term follow-up need to be further investigated.

Our study was not designed to evaluate competing events as death for noncardiovascular causes, but no difference in mortality was found in patients treated or not with corticosteroid, which is in accordance with a recent trial showing no effect on survival in dexamethasone-treated patients.[35,41]

The potential beneficial effect of corticosteroid treatment in term of myocardial injury reduction and MACE risk were present only in patients disclosing hs-cTnT elevation. The finding of our study could have important implications as it suggests the usefulness of glucocorticoids only in a subset of CAP patients. This may be in accordance with previous reports showing that methylprednisolone 0.5 mg/kg twice daily reduces morbidity and mortality only in cases of severe CAP.[42] Thus, we suggest the need of planning randomized controlled trial (RCT) to assess if CAP patients with elevated troponin could have beneficial effect by corticosteroid treatment in terms of myocardial injury reduction, and ultimately MACE risk reduction and overall survival. In this regard we recently proposed an RCT to evaluate the effect of methylprednisolone 0.5 mg/kg twice daily in patients with CAP.[43]

5 | CONCLUSION

In conclusion, in patients with CAP the increase in troponin is a marker of poor outcomes in terms of MACEs. In patients with troponin elevation corticosteroid treatment may protect against myocardial damage and poor cardiovascular outcomes.

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COMPETING INTERESTS

None declared.

CONTRIBUTORS

R.Can., R.Car. and F.V. designed the study, interpreted the results and drafted the manuscript. V.C., C.N., S.B. and G.T. acquired and analysed the data. M.F., C.C. and P.P. analysed the data, interpreted the results and reviewed the manuscript. All authors read and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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