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

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Abstract

Background and Purpose: Patients with community acquired pneumonia (CAP) may suffer from myocardial injury, which is associated with increased risk of major adverse cardiovascular events (MACE). Corticosteroids are often prescribed to CAP patients, but the relationship between their use, myocardial injury and outcomes in these patients is unknown. 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). Patients with hs-cTnT elevation were older, more likely to be former smokers, and with a higher prevalence of cardiovascular comorbidities. In a median follow-up of 22.7 months, a multivariable Cox proportional hazard regression analysis showed age, heart failure and the increasing quintiles of hs-cTnT (HR: 2.16; 95% CI: 1.82-2.58; p<0.001) predicted MACE. In-hospital corticosteroid use was found in 137 (25%) patients. Among patients with hs-cTnT >0.014 μg/L at admission, 102 patients (31%) were on corticosteroids and showed lower intra-hospital hs-cTnT increase compared to untreated ones (p=0.003). Among patients with hs-cTnT >0.014 μg/L, corticosteroid-treated patients showed a lower incidence of MACE than untreated ones [29% (27/99) vs. 43% (92/213); p value =0.042]; no effect of corticosteroids on MACE was observed in CAP patients with normal troponin. Conclusion and Implications: The study provides evidence that corticosteroid use is associated with lower increase of hs-cTnT and incidence of MACE in CAP patients.

1 Introduction

Despite improved management by antibiotic therapy the risk of hospitalization, morbidity and mortality is 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 1,182 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 ECG modification compatible with 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

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the use of corticosteroids in CAP unless of precise indications for their use as in case of coexistent asthma, chronic obstructive pulmonary disease (COPD) or autoimmune diseases.[10]

Corticosteroids seem to have also 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 non-users.[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 (MACE).

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 3 medical centres from the University-Hospital Policlinico Umberto I, Sapienza University of Rome, Italy: Department of Internal Medicine and Medical Specialties, Department of Clinical Medicine, Department of Public Health and Infectious Diseases).

We enrolled adults who met the following criteria: 1) age [?]18 years; 2) clinical presentation of an acute illness with at least two or more of the signs or symptoms of CAP, as previously reported 4 and 3) presence of new consolidation(s) on chest X-ray.[12] Pneumonia was defined as CAP diagnosed upon hospitalization in a patient who did not meet the criteria for healthcare-associated pneumonia.[13]

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,[14] presence of malignancy; pregnancy or breastfeeding; documented severe allergy to antibiotics; healthcare-associated pneumonia.[13]

All patients with CAP admitted to the 3 units after giving written informed consent from October 2011 to January 2016, 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.[15] For each patient recruited, 12-lead electrocardiogram (ECG) and routine blood laboratory tests were immediately performed after admission. Hs-cTnT were 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.[16]

2.1.2 In-hospital outcomes

The occurrence of a MI was diagnosed according to the Third Universal Definition of Myocardial Infarction.[17] ST-elevation MI (STEMI) and non-ST-elevation MI (NSTEMI) were defined as previously reported[17] 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

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investigation/procedure/operation; death due to other specified cardiovascular causes.

2.1.3 Long-term follow-up

Follow-up data about MACE (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 was unaware of the clinical and laboratory characteristics of any patient.

2.2 Isolation of human peripheral blood mononuclear cells (PBMCs)

Freshly taken EDTA-blood from healthy subjects (HS) was diluted with PBS (1:4), stratified over 10 mL of Ficoll-Paque and then centrifuged at 1660 rpm for 30' at 20 °C. The mononuclear cell layer was aspired and transferred into a 50 mL conical tube and washed two times with PBS by centrifugation at 1000 g for 10'. The cell pellet was suspended in 1 ml of PBS and was pre-incubated (20 minutes at 37°C) with scalar concentration (150-300-600 ng/ml) of methylprednisolone (Sigma Aldrich, USA) or betamethasone (Sigma Aldrich, USA), which corresponded to the levels detected in serum of patients upon glucocorticoid administration.[18] After incubation, samples were stimulated with LPS (40 pg/ml; Sigma Aldrich, USA) for 10' at 37°C. Cells were centrifuged for 3' at 3000 rpm. Supernatants and pellet were stored at -80 degC 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.[19] Values were expressed as pg/ml; 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) was evaluated by a Colorimetric Detection Kit (Arbor Assays, MI, USA) and expressed as μM. 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, USA). Equal amounts of protein (30 μg/lane) were solubilized in a 2X Leammli sample buffer, separated on a 10-12% SDS-polyacrylamide gel and then electrotransferred to nitrocellulose membranes. After blocking with Bovine Serum Albumin (BSA, 5%; Sigma Aldrich, USA) the membranes were incubated overnight at 4°C with rabbit polyclonal anti-p-p47phox (Abcam, UK), anti-p-AKT and anti-β-Actin antibody (Santa Cruz Biotechnology, USA) and subsequently with secondary antibody (1:3000; Bio-Rad, USA). The immune complexes were detected by enhanced chemiluminescence substrate (ECL Substrates, Bio-Rad, USA). Densitometric analysis of the bands was performed using Image J software.

2.6 Serum hs-cTnT measurement

Hs-cTnT levels were measured using the Elecsys 2010 (Roche Diagnostics, Indianapolis, Indiana) at a dedicated core laboratory. According to the manufacturer, the 99^{th}-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.[20]

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.

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Data were analyzed 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 <0.05 were considered statistically significant. All tests were 2-tailed, and analyses were performed using computer software packages (R version 2.15.2, R Development Core Team, Wien, Austria).

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, AF, compared to patients with normal hs-cTnT. Moreover, they were more likely to be treated with aspirin, thienopyridines, anticoagulants and statins, and belonged 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, however, 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 value <0.001) that was reached from 24 to 72 h from hospital admission, to 0.030 [0.016-0.054] μg/L (p value <0.001) 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 (not shown).

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

Among the entire population, 29 patients were lost to follow-up because they refused to participate to the follow-up or did not answer to telephone calls. Follow-up data were, therefore, available in 512 patients, who 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 non-fatal MI, 74 cardiovascular deaths).

In the whole cohort, a multivariable COX regression analysis showed that age (HR: 1.06; 95%CI: 1.04-1.08; p value <0.001); in-hospital maximum levels of hs-cTnT (HR: 2.02; 95% CI: 1.59-2.58; p<0.001), heart failure (1.90; 95% CI: 1.23-2.95; p=0.004), and CHD (HR: 1.65; 95% C.I. 1.09-2.49; p value =0.018) independently predicted MACE, 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 were stratified in 5 quintiles ([?]0.010 μg/L; 0.011-0.014 μg/L; 0.015-0.030 μg/l; 0.031-0.074 μg/L, >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 value <0.001) predicted MACE, after adjusting for the possible confounding variable (Figure 1).

3.2 Systemic corticosteroids and troponins.

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In the whole cohort, 137 patients (25%) were treated with systemic corticosteroids during the in-hospital stay. Corticosteroid treatment encompassed methylprednisolone (60%; 20-80 mg/day), betamethasone (22%; 4-8 mg/day) and prednisone (18%; 25-50 mg/day).

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 ones (Table 2).

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 non-treated ones (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, respectively]; p value =0.646). 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 value <0.001 and 0.050 [0.027-0.108] μg/L; p<0.001, respectively); however, the increase in hs-cTnT was significantly lower in the corticosteroid-treated patients compared to untreated ones (0.006 [0.00-0.019] vs. 0.012 [0.002-0.040] μg/L; p value =0.003). At discharge, either group showed similar hs-cTnT levels. A MANOVA analysis confirmed an effect of corticosteroids on hs-cTnT, also after adjusting for age, coronary heart disease, heart failure and COPD (F=4.1; p=0.021) (Figure 2). Among patients with hs-cTnT [?]0.014, no significant differences in hs-cTnT levels were found between patients treated or not with corticosteroids, during the in-hospital stay and at discharge (not shown).

3.3 Corticosteroids and MACE at long-term follow-up.

Among patients with hs-cTnT >0.014 μg/L, corticosteroid-treated patients showed a lower incidence of MACE than untreated ones [29% (29/99) vs. 43% (92/213); p=0.042] during the follow-up (Figure 3).

A COX regression analysis confirmed that in-hospital corticosteroid use was inversely associated to MACE (HR: 0.64; 95% CI: 0.41-0.97; p=0.038), while age (HR: 1.05; 95% CI: 1.03-1.08;p<0.001); CHD (HR: 1.6; 95% CI: 1.05-2.33; p=0.029); congestive heart failure (HR: 1.72; 95% CI: 1.15-2.58; p value =0.009) were positively associated to MACE, 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, the incidence of MACE did not differ in patients treated or not with corticosteroids (14% vs. 7%; p value =0.222).

3.4 Corticosteroids and sNOX2-dp

At hospital admission, patients disclosed elevated levels of serum sNOX2-dp, that correlated with baseline hs-cTnT (Rs: 0.464; p value <0.001) and with maximum levels of hs-cTnT (Rs: 0.525; p value <0.001); sNox2 decreased at hospital discharge (27 [17-41] vs. 22[15-33] pg/ml; p value <0.001).

A MANOVA analysis showed that sNox2-dp decreased over time more in corticosteroid-treated patients compared to not-treated ones, also after adjusting for possible confounding factors (age, PSI classes, COPD, AF, smoking habit) (F=7.9; p value =0.005) (Figure 4A).

After dividing the whole cohort in 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=0.012) (Figure 4B) but not in patients with hs-cTnT[?]0.014 μg/L (F=1.3; p=0.261) (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 of 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).

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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).

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 up-regulation.[21] Betamethasone and methylprednisolone-treated cells stimulated with LPS showed a significant decrease of AKT phosphorylation compared to LPS-treated cells alone (Figures 6, A and B). This effect was dose-dependent with a significance reached at concentrations of 300 and 600 ng/ml compared to 150 ng/ml (Figure 6, A and 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 6, C and 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 MACE in a long-term follow-up. The effect of corticosteroids seems to be mediated by an antioxidant mechanism related to Nox2 down-regulation.

Previous studies showed that myocardial injury, as assessed by cardiac troponins 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; i.e. over the 99^{th}percentile) were found in more than 50% of patients and associated to in hospital MI in about 10% of patients.[5] The increase of hs-cTnT was mostly detected within 24 -72 h 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 SMT 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.[22] Recently, in a cohort of 730 CAP patients followed-up for one year, Menéndez R found that hs-cTnT elevation at admission independently predicted early but not late cardiovascular events.[23] 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 (i.e. at 24-72 hours) were a strong predictor of MACE 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.[11] However, no data exist about the potential effect 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 MACE incidence at a 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 to Nox2 up-regulation in patients with CAP suggesting that Nox2-dependent oxidative stress is implicated in myocardial injury.[24] 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.[25] Numerous studies indicated that Nox2 localizes in cardiomyocytes where it could play an important role in redox balance,[26,27] suggesting that enhanced Nox2-derived oxidative stress may be implicated in myocardial

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injury. Overactivation of Nox2 in CAP patients may be explained by previous report 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 knock-out cells.[28]

Then, 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 ones; 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_2production in LPS stimulated cells, through a reduction in AKT and $p47^{phox}$ phosphorylation.

The present study has limitation and implication. The study supports and extends previous reports indicating that patients with troponin elevation are at higher risk of long-term MACE. 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. Corticosteroids were administered at the discretion of the managing physician for perceived need. However, corticosteroid-treated patients were older and with more commorbidities like COPD; thus this fact should reinforce the results of the present report. Even if the herewith reported antioxidant effect and previous reports showing glucocorticoids' antiplatelet effects[18] could explain the cardiovascular protection, the reasons for MACE reduction during the long-term follow-up need to be further investigated.

The beneficial effect of corticosteroid treatment in term of myocardial injury reduction and MACE risk were present only in patients disclosing hs-cTnT elevation. This finding 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 corticosteroids reduce morbidity and mortality only in case of severe CAP.[29] Thus, we suggest the need of planning 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.

Nox2 overactivation in CAP patients might have important implications not only in CAP patients but also in other infections mediated by RNA viruses such as COVID-19. Thus, COVID-19 patients often present elevated troponin levels, which are associated with poor outcomes.[30] Due to the role of Nox2 in the pathogenesis of RNA viruses, our findings suggest to evaluate if Nox2 activation is implicated in the pathogenicity of COVID-19; in fact, Nox2 oxidase activation down-regulates antibody production, that is required for virus clearance.[28]

4.1Conclusion.

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

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

References

1. Torres A, Peetermans WE, Viegi G, Blasi F. Risk factors for community-acquired pneumonia in adults in Europe: A literature review. *Thorax* 2013; **68** :1057–1065.

2. Violi F, Cangemi R, Calvieri C. Pneumonia, thrombosis and vascular disease. *J Thromb Haemost* 2014;**12** :1391-400.

3. Corrales-Medina VF, Musher DM, Shachkina S, Chirinos JA. Acute pneumonia and the cardiovascular system. *Lancet*2013;**381** :496-505.

4. Violi F, Cangemi R, Falcone M, Taliani G, Pieralli F, Vannucchi V, Nozzoli C, Venditti M, Chirinos JA, Corrales-Medina VF; SIXTUS (Thrombosis-Related Extrapulmonary Outcomes in Pneumonia) Study Group. Cardiovascular Complications and Short Term Mortality Risk in Community-Acquired Pneumonia. Clin Infect Dis. 2017;**64** :1486-1493.

5. Cangemi R, Casciaro M, Rossi E, Calvieri C, Bucci T, Calabrese CM, Taliani G, Falcone M, Palange P, Bertazzoni G, Farcomeni A, Grieco S, Pignatelli P, Violi F, Albanese F, Biliotti E, Carnevale R, Catasca E, Celestini A, Esvan R, Fazi L, Marinelli P, Mordenti M, Napoleone L, Palumbo M, Pastori D, Perri L, Proietti M, Marco RC, Russo A, et al. Platelet activation is associated with myocardial infarction in patients with pneumonia. *J Am Coll Cardiol* 2014;**64** :1917–1925.

6. Stern A, Skalsky K, Avni T, Carrara E, Leibovici L, Paul M. Corticosteroids for pneumonia. *Cochrane Database Syst. Rev.*2017;**12** :CD007720.

7. Jiang S, Liu T, Hu Y, Li R, Di X, Jin X, Wang Y, Wang K. Efficacy and safety of glucocorticoids in the treatment of severe community-acquired pneumonia: A meta-analysis. *Medicine (Baltimore)*2019;**98** :e16239.

8. Feldman C, Anderson R. Corticosteroids in the adjunctive therapy of community-acquired pneumonia: An appraisal of recent meta-analyses of clinical tria. *J Thorac Dis* 2016;**8** :E162-171.

9. Wan YD, Sun TW, Liu ZQ, Zhang SG, Wang LX, Kan QC. Efficacy and safety of corticosteroids for community-Acquired pneumonia: A systematic review and meta-Analysis. *Chest* ; 2016;**149** :209–219.

10. Metlay JP, Waterer GW, Long AC, Anzueto A, Brozek J, Crothers K, Cooley LA, Dean NC, Fine MJ, Flanders SA, Griffin MR, Metersky ML, Musher DM, Restrepo MI, Whitney CG. Diagnosis and treatment of adults with community-acquired pneumonia. *Am J Respir Crit Care Med*2019;**200** :E45–E67.

11. Cangemi R, Falcone M, Taliani G, Calvieri C, Tiseo G, Romiti GF, Bertazzoni G, Farcomeni A, Violi F, Battaglia S, Biliotti E, Calabrese CM, Celestini A, Casciaro M, Angelis M De, Diego I Di, Marzio P De, Esvan R, Ferraro G, Sulekova LF, Franchi C, Giordo L, Khoury F, Morelli S, Catassi GN, Palange P, Pastori D, Prosperi A, Raparelli V, Capparuccia MR, et al. Corticosteroid use and incident myocardial infarction in adults hospitalized for community-acquired pneumonia. *Ann Am Thorac Soc* 2019;**16** :91–98.

12. Bonten MJM, Huijts SM, Bolkenbaas M, Webber C, Patterson S, Gault S, Werkhoven CH Van, Deursen AMM Van, Sanders EAM, Verheij TJM, Patton M, McDonough A, Moradoghli-Haftvani A, Smith H, Mellelieu T, Pride MW, Crowther G, Schmoele-Thoma B, Scott DA, Jansen KU, Lobatto R, Oosterman B,

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Visser N, Caspers E, Smorenburg A, Emini EA, Gruber WC, Grobbee DE. Polysaccharide conjugate vaccine against pneumococcal pneumonia in adults. *N Engl J Med* 2015;**372** :1114–1125.

13. Falcone M, Venditti M, Shindo Y, Kollef MH. Healthcare-associated pneumonia: Diagnostic criteria and distinction from community-acquired pneumonia. *Int J Infect Dis* 2011;**15** .

14. Mandell LA, Wunderink RG, Anzueto A, Bartlett JG, Campbell GD, Dean NC, Dowell SF, File TM, Musher DM, Niederman MS, Torres A, Whitney CG. Infectious Diseases Society of America/American Thoracic Society Consensus Guidelines on the Management of Community-Acquired Pneumonia in Adults. *Clin Infect Dis* 2007;**44** :S27–S72.

15. Aujesky D, Fine MJ. The Pneumonia Severity Index: A Decade after the Initial Derivation and Validation. *Clin Infect Dis*2008;**47** :S133–S139.

16. Piepoli MF, Hoes AW, Agewall S, Albus C, Brotons C, Catapano AL, Cooney M-T, Corrà U, Cosyns B, Deaton C, Graham I, Hall MS, Hobbs FDR, Løchen M-L, Löllgen H, Marques-Vidal P, Perk J, Prescott E, Redon J, Richter DJ, Sattar N, Smulders Y, Tiberi M, Worp HB van der, Dis I van, Verschuren WMM, Binno S, ESC Scientific Document Group. 2016 European Guidelines on cardiovascular disease prevention in clinical practice.*Eur Heart J* 2016;**37** :2315–2381.

17. Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD, Katus HA, Apple FS, Lindahl B, Morrow DA, Clemmensen PM, Johanson P, Hod H, Underwood R, Bax JJ, Bonow RO, Pinto F, Gibbons RJ, Fox KA, Atar D, Newby LK, Galvani M, Hamm CW, Uretsky BF, Steg PG, Wijns W, Bassand JP, Menasché P, Ravkilde J, Ohman EM, et al. Third universal definition of myocardial infarction. *Circulation* 2012;**126** :2020–2035.

18. Cangemi R, Carnevale R, Nocella C, Calvieri C, Cammisotto V, Novo M, Castellani V, D'Amico A, Zerbinati C, Stefanini L, Violi F, SIXTUS Study Group. Glucocorticoids impair platelet thromboxane biosynthesis in community-acquired pneumonia. *Pharmacol Res*2018;**131** :66–74.

19. Carnevale R, Silvestri R, Loffredo L, Novo M, Cammisotto V, Castellani V, Bartimoccia S, Nocella C, Violi F. Oleuropein, a component of extra virgin olive oil, lowers postprandial glycaemia in healthy subjects. *Br J Clin Pharmacol* 2018;**84** :1566–1574.

20. Reichlin T, Hochholzer W, Bassetti S, Steuer S, Stelzig C, Hartwiger S, Biedert S, Schaub N, Buerge C, Potocki M, Noveanu M, Breidthardt T, Twerenbold R, Winkler K, Bingisser R, Mueller C. Early diagnosis of myocardial infarction with sensitive cardiac troponin assays. *N Engl J Med* Massachussetts Medical Society; 2009;**361** :858–867.

21. Chen Q, Powell DW, Rane MJ, Singh S, Butt W, Klein JB, McLeish KR. Akt Phosphorylates p47 phox and Mediates Respiratory Burst Activity in Human Neutrophils . *J Immunol* The American Association of Immunologists; 2003;**170** :5302–5308.

22. Vestjens SMT, Spoorenberg SMC, Rijkers GT, Grutters JC, Berg JM Ten, Noordzij PG, Garde EMW van de, Bos WJW, Biesma DH, Endeman H, Hardeman H, Heijligenberg R, Meijvis SCA, Remmelts HH, Velzen-Blad H van, Voorn GP (Paul. High-sensitivity cardiac troponin T predicts mortality after hospitalization for community-acquired pneumonia. *Respirology*2017;**22** :1000–1006.

23. Menéndez R, Méndez R, Aldás I, Reyes S, Gonzalez-Jimenez P, España PP, Almirall J, Alonso R, Suescun M, Martinez-Dolz L, Torres A. Community-Acquired Pneumonia Patients at Risk for Early and Long-term Cardiovascular Events Are Identified by Cardiac Biomarkers. *Chest*2019;**156** :1080–1091.

24. Cangemi R, Calvieri C, Bucci T, Carnevale R, Casciaro M, Rossi E, Calabrese CM, Taliani G, Grieco S, Falcone M, Palange P, Bertazzoni G, Celestini A, Pignatelli P, Violi F. Is NOX2 Upregulation Implicated in Myocardial Injury in Patients with Pneumonia? *Antioxid Redox Signal* 2014;**20** :2949–2954.

25. Zhang Y, Tocchetti CG, Krieg T, Moens AL. Oxidative and nitrosative stress in the maintenance of myocardial function. *Free Radic Biol Med.* 2012;**53** :1531-1540.

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26. Sirker A, Murdoch CE, Protti A, Sawyer GJ, Santos CXC, Martin D, Zhang X, Brewer AC, Zhang M, Shah AM. Cell-specific effects of Nox2 on the acute and chronic response to myocardial infarction. *J Mol Cell Cardiol* 2016;**98** :11–17.

27. Zhang M, Perino A, Ghigo A, Hirsch E, Shah AM. NADPH oxidases in heart failure: Poachers or gamekeepers? *Antioxid Redox Signal*2013;**18** :1024-1241.

28. To EE, Vlahos R, Luong R, Halls ML, Reading PC, King PT, Chan C, Drummond GR, Sobey CG, Broughton BRS, Starkey MR, Sluis R Van Der, Lewin SR, Bozinovski S, O'Neill LAJ, Quach T, Porter CJH, Brooks DA, O'Leary JJ, Selemidis S. Endosomal NOX2 oxidase exacerbates virus pathogenicity and is a target for antiviral therapy. *Nat Commun.*2017;**8** :69.

. 29. Torres A, Sibila O, Ferrer M, Polverino E, Menendez R, Mensa J, Gabarrús A, Sellarés J, Restrepo MI, Anzueto A, Niederman MS, Agustí C. Effect of corticosteroids on treatment failure among hospitalized patients with severe community-acquired pneumonia and high inflammatory response: A randomized clinical trial. *JAMA*2015;**313** :677–686.

30. Clerkin KJ, Fried JA, Raikhelkar J, Sayer G, Griffin JM, Masoumi A, Jain SS, Burkhoff D, Kumaraiah D, Rabbani LR, Schwartz A, Uriel N. Coronavirus Disease 2019 (COVID-19) and Cardiovascular Disease.*Circulation* 2020;**141** :1648-1655.

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)*	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

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	Total patients	Patients with hs-cTnT[?]0.014 µg/L	Patients with hs-cTnT>0.014 µg/L	P value
Statins (%)	30	23	34	0.005

Legend: AF: atrial fibrillation; COPD: chronic obstructive pulmonary disease; OAC: oral anticoagulants, PSI: pneumonia severity index. * data are expressed as mean ±standard deviation.

Table 2. Clinical characteristics of patients treated or not with systemic corticosteroids.

	Total patients	Not corticosteroids-treated	Corticosteroid-treated	P value
N.	541	403	138	
Age (years)	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

Legend: AF: atrial fibrillation; COPD: chronic obstructive pulmonary disease; OAC: oral anticoagulants, PSI: pneumonia severity index. * data are expressed as mean ±standard deviation.

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

	Non-corticosteroid group	Corticosteroids group	P value
N.	216	102	
Age (years)	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

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	Non-corticosteroid group	Corticosteroids group	P value
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

Legend: AF: atrial fibrillation; COPD: chronic obstructive pulmonary disease; OAC: oral anticoagulants, PSI: pneumonia severity index. * data are expressed as mean ±standard deviation.

Figure legends.

Figure 1. Cumulative MACE survival probability at the long-term follow-up, according to the quintiles of maximum levels hs-cTnT during the in-hospital stay. HR: 2.16; 95% CI: 1.82-2.58; p value <0.001 for each increasing quintile (multivariable COX regression analysis).

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 MACE according to in-hospital corticosteroid treatment among patients with hs-cTnT > 0.014 μg/L during the in-hospital stay (log-rank test).

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).

Figure 5. Glucocorticoids and oxidative stress. Nox2 activation (A) and H2O2 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 (40pg/ml) (n = 5 experiments) (*p value < 0.05; **p value <0.001;***p value <0.0001; Kruskal–Wallis test).

Figure 6. Glucocorticoids and pathway of oxidative stress. AKT (A) and p47phox (C) phosphorylation were analyzed in in human peripheral blood mononuclear cells (PBMCs) incubated with scalar concentrations (150–600 ng/ml) of betamethasone or methylprednisolone and stimulated with LPS (40pg/ml) (n = 3 experiments) (*p value < 0.05; **p value <0.001;***p value <0.0001; 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).

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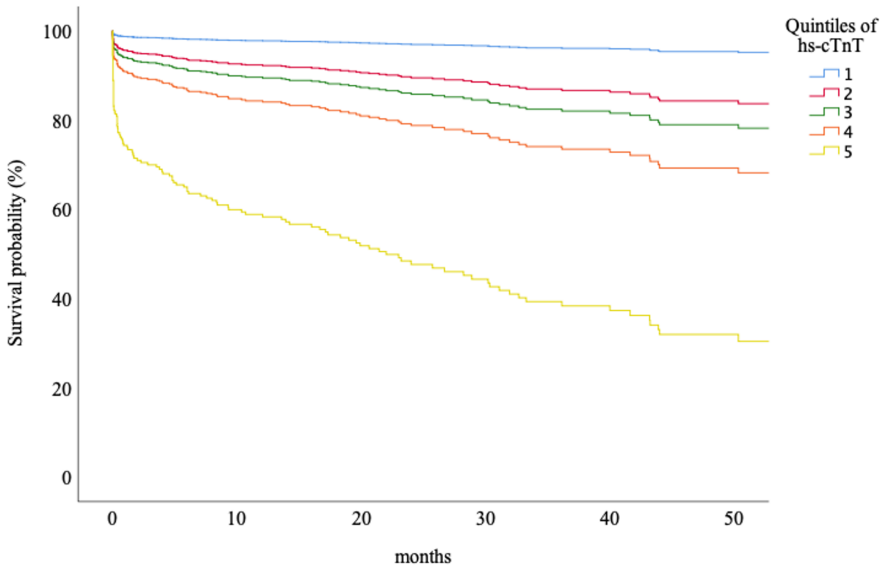

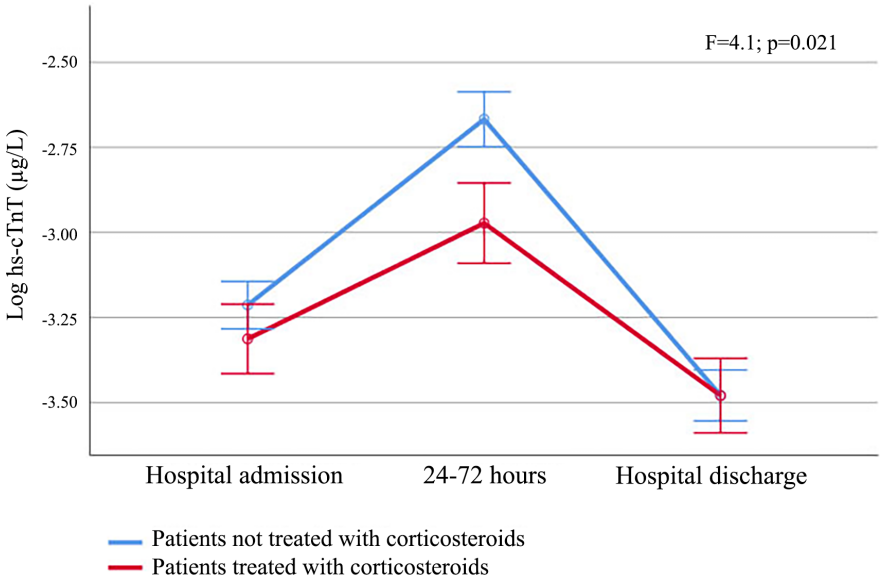

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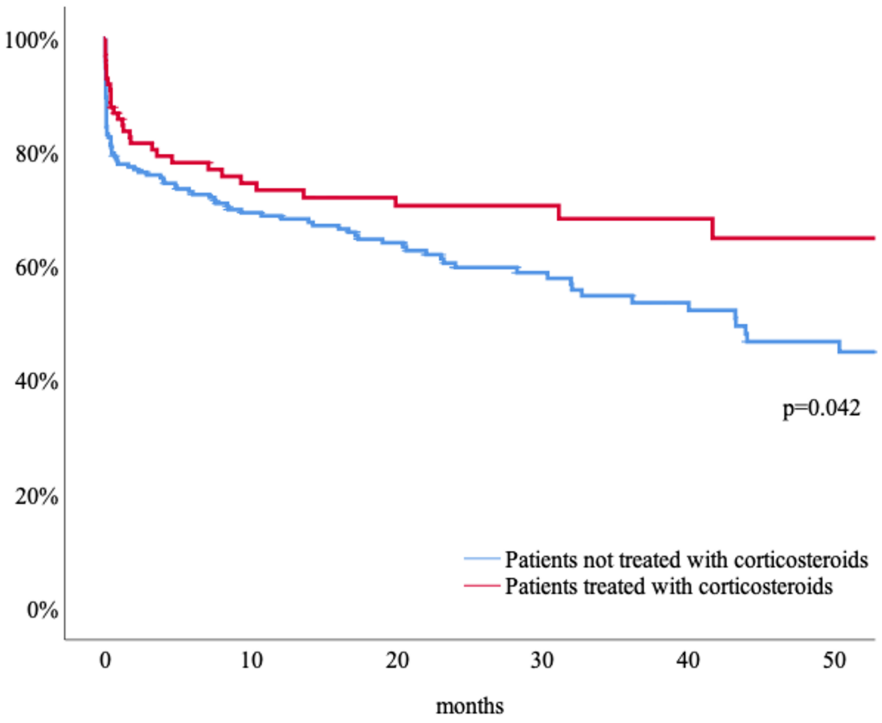

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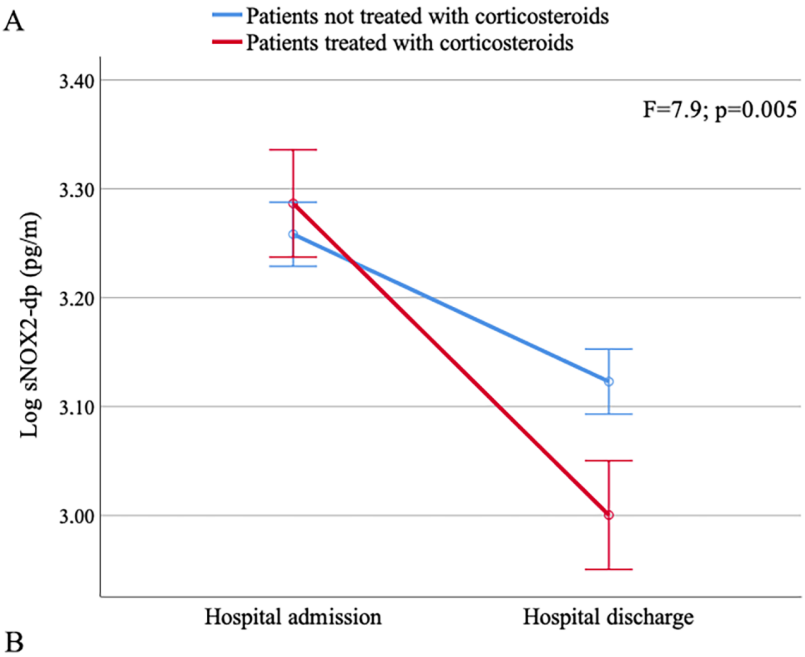
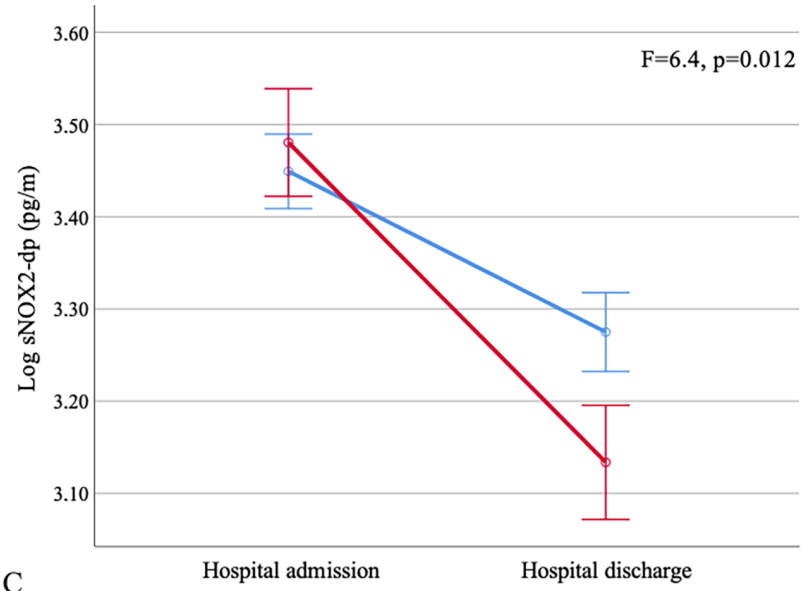
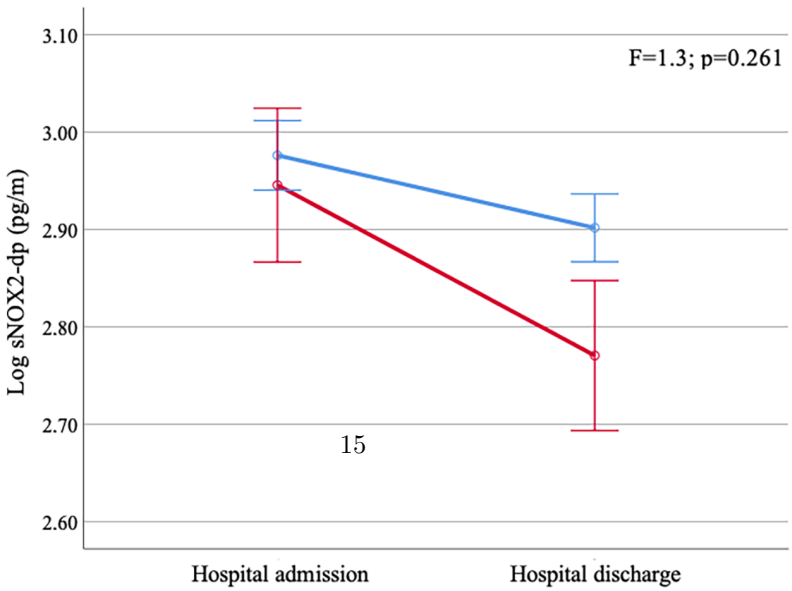
Panel A



Panel B



Panel C



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