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Original Article

Impaired flow-mediated dilation in hospitalized patients with community-acquired pneumonia

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ABSTRACT

Background: Community-acquired pneumonia (CAP) is complicated by cardiovascular events as myocardial infarction and stroke but the underlying mechanism is still unclear. We hypothesized that endothelial dysfunction may be implicated and that endotoxemia may have a role.
Methods: Fifty patients with CAP and 50 controls were enrolled. At admission and at discharge, flow-mediated dilation (FMD), serum levels of endotoxins and oxidative stress, as assessed by serum levels of nitrite/nitrate (NOx) and isoprostanes, were studied.
Results: At admission, a significant difference between patients with CAP and controls was observed for FMD (2.1 ± 0.3 vs 4.0 ± 0.3%, $p < 0.001$), serum endotoxins (157.8 ± 7.6 vs 33.1 ± 4.8 pg/ml), serum isoprostanes (341 ± 14 vs 286 ± 10 pM, $p = 0.009$) and NOx (24.3 ± 1.1 vs 29.7 ± 2.2 μM). Simple linear correlation analysis showed that serum endotoxins significantly correlated with Pneumonia Severity Index score ($Rs = 0.386$, $p = 0.006$). Compared to baseline, at discharge CAP patients showed a significant increase of FMD and NOx (from 2.1 ± 0.3 to 4.6 ± 0.4%, $p < 0.001$ and from 24.3 ± 1.1 to 31.1 ± 1.5 μM, $p < 0.001$, respectively) and a significant decrease of serum endotoxins and isoprostanes (from 157.8 ± 7.6 to 55.5 ± 2.3 pg/ml, $p < 0.001$, and from 341 ± 14 to 312 ± 14 pM, $p < 0.001$, respectively). Conversely, no changes for FMD, NOx, serum endotoxins and isoprostanes were observed in controls between baseline and discharge. Changes of FMD significantly correlated with changes of serum endotoxins ($Rs = -0.315$; $p = 0.001$).
Conclusions: The study provides the first evidence that CAP is characterized by impaired FMD with a mechanism potentially involving endotoxin production and oxidative stress.

1. Introduction

Community-acquired pneumonia (CAP) is the most common infection leading to hospitalization in intensive care units and the most common cause of death associated with infectious disease [1].

Epidemiological studies have shown that respiratory tract infections are associated with an increased risk for cardiovascular events such as

Abbreviation: CAP, community-acquired pneumonia; FMD, flow-mediated dilation; NO, nitric oxide; NOx, nitrite/nitrate; MI, myocardial infarction; NSTEMI, non-ST elevation myocardial infarction; STEMI, ST-elevation MI; T2DM, type 2 diabetes mellitus; LPS, lipopolysaccharides; CHF, congestive heart failure; PSI, Pneumonia Severity Index.

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acute myocardial infarction (MI), stroke and cardiac arrhythmia such as atrial fibrillation [2,3]. The relationship between CAP and cardiovascular events is corroborated by studies indicating that influenza vaccination lowers the risk for CAP hospitalization, heart disease, cerebrovascular disease and death from any cause during flu seasons in the elderly [4]. The mechanism accounting for the development of MI in the early phase of CAP is still unclear [2,5]. Platelets have been suggested to play a role as markers of in vivo platelet activation; soluble CD40 Ligand and P-selectin have been associated with an enhanced risk of MI [6]. Furthermore, an observational study demonstrated that CAP patients treated with aspirin had a lower risk of experiencing MI during a 30-day follow-up compared to non aspirin users [7]. Changes in artery vasodilation could be another mechanism accounting for MI occurrence. Thus, we have recently showed that CAP is essentially associated with non-ST elevation myocardial infarction (NSTEMI), suggesting that incomplete coronary occlusion or enhanced oxygen demand could account for the increased risk of MI in CAP patients [6]. Accordingly, we speculated that impaired artery dilation could complicate the clinical course of CAP and potentially precipitate in acute coronary syndrome. Flow-mediated dilation (FMD) is an established marker of artery dilation, which is associated with cardiovascular outcomes; [8,9] thus, impaired FMD increases the risk for cardiovascular disease [8,9]. To address if acute phase of CAP is associated with impaired artery vasodilation, FMD was measured at admission and at discharge in patients with CAP and in hospitalized patients with clinical disease unrelated to any infection. Furthermore, to investigate the underlying mechanism, we explored the interplay among endotoxemia, oxidative stress and FMD.

2. Methods

2.1. Patients

In this cross-sectional study, we included 50 consecutive patients with CAP recruited between October 2014 and March 2015 at the Internal Medicine ward of "Sapienza" University of Rome. All patients admitted to the medical ward with diagnosis of CAP through the emergency department were consecutively recruited. Patients who fulfilled the following criteria were enrolled in the study after giving written informed consent: (1) age 18 years or over; (2) clinical presentation of an acute disease with 1 or more of the following signs or symptoms suggesting pneumonia: presence of rales, bronchial breath sounds, rhonchi, tachycardia, fever (>38.0 °C), dyspnea, chills, coughing, or chest pain; and (3) presence of new consolidation(s) on chest X-ray. Pneumonia was considered as CAP if it was diagnosed upon hospitalization and the patient had not been discharged from an acute care facility within 14 days preceding the clinical presentation. Patients were excluded from the study if any of the following criteria applied: criteria for health care-associated pneumonia [10], radiographic evidence of a preexisting infiltrates, presence of malignancy**,** pregnancy or breastfeeding, documented severe allergy to antibiotics, or refusal to sign informed consent.

In the same period of CAP enrollment, 50 hospitalized patients without acute infections and matched for sex, age, and comorbidities including diabetes, dyslipidemia, hypertension, chronic obstructive pulmonary disease (COPD), congestive heart failure (CHF) and renal failure were used as controls. Frequency matching procedures were applied to select controls. Matched controls were selected such that the distribution of the relevant characteristics in this group was similar to the distribution in the cases. Fifty out of 532 patients hospitalized between October 2014 and March 2015 were selected. They were hospitalized for CHF (n = 10), syncope (n = 4), hypertension crisis (n = 4), COPD exacerbation (n = 16), decompensated diabetes mellitus (n = 9), new onset arrhythmias (n = 5), and renal failure (n = 2).

Baseline treatments were defined according to patients' pharmacological histories.

Severity of disease at presentation was assessed by the Pneumonia Severity Index (PSI), a validated prediction score for 30-day mortality in patients with CAP [11,12].

Systemic inflammatory response syndrome (SIRS), was defined as previously described [13], i.e. the occurrence of at least two of the following criteria: fever >38.0 °C or hypothermia <36.0 °C, tachycardia >90 beats/min, tachypnea >20 breaths/min, leukocytosis >12*109/l or leucopoenia <4*109/L. Sepsis was defined as SIRS in addition to a documented or presumed infection [13].

Type 2 diabetes mellitus (T2DM), hypertension, history of coronary heart disease, dyslipidemia, CHF and COPD were defined as previously described [14–16]. ST-elevation MI (STEMI) and NSTEMI were defined as previously reported [17] and were confirmed by cardiologists.

Daily diet was based on the hospital guidelines and was tailored according to age, nutritional status and severity of the comorbidities.

In all subjects we performed FMD and collected blood samples to analyze markers of oxidative stress as assessed by serum isoprostanes, nitrite/nitrate (NOx), and serum endotoxins (Lipopolysaccharides, LPS) at admission and at discharge (10 ± 3 days in average). Given that dietary changes could potentially influence serum NOx [18], we paid attention that the daily diet of each patient was not modified during the intra-hospital stay.

This study was conducted according to the principles stated in the Declaration of Helsinki. The institutional review board approved this prospective, observational study, which was registered at ClinicalTrials.gov (Identifier: NCT01773863).

2.2. FMD

Ultrasound assessment of basal brachial diameter and endothelial dependent FMD of brachial artery were investigated according to current guidelines [19] and as previously described [20]. FMD was performed in all patients by the same operator. To evaluate the reproducibility of FMD ten hospitalized patients underwent FMD measurement on 2 separate occasions (baseline AND after 1 week). Variability of different measurements was assessed by using intra-class correlation coefficient (ICC). ICC for brachial diameter at rest and FMD was 0.98 and 0.89, respectively.

2.3. Blood sampling

Blood samples were collected between 8.00 and 9.00 am for routine biochemical evaluation, including fasting total cholesterol and glucose, and for oxidative stress analysis. Blood samples were collected in Vacutainers (Vacutainer Systems, Belliver Industrial Estate, Plymouth, UK) after an overnight fast (12 h). Samples were centrifuged at 300 *g* for 10 min and the supernatant was collected and stored at −80 °C until dosage.

2.4. Serum isoprostanes (8-iso-PGF2α-III) assays

Analysis of isoprostanes was performed measuring serum 8-iso-PGF2α-III by a validated enzyme immunoassay method (Cusabio). Values were expressed as pM; intra-assay and inter-assay coefficients of variation were 5.8% and 5.0%, respectively.

2.5. Serum nitrite and nitrate (NOx)

NOx were assessed in serum by the measurements of metabolic end-products i.e. (Tema Ricerca). Intra- and inter-assay coefficients of variation were 2.9% and 1.7%, respectively.

2.6. Endotoxemia

Serum levels of endotoxins (Lipopolysaccharides, LPS) were measured by a validated enzyme immunoassay method (Cusabio). Briefly,

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