Oxidative stress in immunocompetent patients with severe community-acquired pneumonia. A pilot study

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Abstract
Objective: A comparison was made of the oxidative stress (OS) levels of patients with either viral or bacterial severe community-acquired pneumonia (sCAP) and of patients without infection (healthy volunteers [HV]) and patients with acute myocardial infarction (AMI)).

Design: A prospective observational study was made.

Patients: Critically ill patients with sCAP.

Variables: The TBARS level was measured as an index of oxidative injury. SOD, CAT and redox glutathione system (GSH, GSSG, GR, GPx) activities were measured as reflecting antioxidant capacity. Severity of illness was assessed by the APACHE II, SOFA and SIRS scores.

Results: Thirty-seven subjects were included: 15 patients with CAP (12 of bacterial origin [BCAP] and 3 due to 2009 A/H1N1 virus [VCAP]), 10 HV and 12 AMI patients. Intensive care CAP mortality was 26.7% (n = 4). Plasmatic TBARS levels were higher in CAP patients than in HV, but similar to those recorded in AMI patients. In contrast, VCAP was associated with lower TBARS levels, and some components of the glutathione redox system were higher in BCAP patients and HV. The OS levels did not differ between survivors and non-survivors.

Conclusion: Our results suggest the occurrence of higher OS in sCAP patients compared with HV. In contrast, lower TBARS levels were observed in VCAP patients, suggesting an increase of antioxidant activity related to the redox glutathione system. However, further research involving a larger cohort is needed in order to confirm these findings.

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Keywords
Oxidative stress; 2009 Influenza A/H1N1 virus; Bacterial pneumonia; Community-acquired pneumonia
Estrés oxidativo en pacientes inmunocompetentes con neumonía comunitaria grave: Un estudio piloto

Resumen

Objetivos: Comparar el estrés oxidativo (EO) en pacientes con neumonía comunitaria grave (NCG) según su etiología y respecto de voluntarios sanos (VS) y pacientes con infarto agudo de miocardio (IAM).

Diseño: Estudio prospectivo, observacional.

Pacientes: Pacientes con NCG ingresados en unidades de cuidados intensivos.

Variables: Los niveles de lipoperoxidación (TBARS) fueron considerados como índice de oxidación, mientras que SOD, CAT y la actividad del sistema redox-glutation (GSH, GSSG, GR, GPx) fueron considerados capacidad antioxidante. La gravedad de los pacientes fue valorada mediante las escalas APACHE II, SOFA y SIRS.

Resultados: Treinta y siete sujetos fueron incluidos, 15 pacientes con NCG (12 con etiología bacteriana [NB] y 3 viral 2009 A/H1N1 [NV]), 10 VS y 12 con IAM. La mortalidad global fue del 26,7% (n = 4). Los TBARS plasmáticos fueron superiores en NCG respecto de VS, pero similares al IAM. En contraste, la NV se asoció con menores niveles de TBARS e incremento de componentes del sistema redox-glutation respecto de NB y voluntarios sanos. No se observó asociación entre mortalidad y EO.

Conclusión: Nuestros resultados evidencian la presencia de EO en pacientes con NCG respecto de los controles. En contraste, la evidencia de un menor nivel de TBARS en la NV respecto de los VS sugiere un incremento de la actividad antioxidante relacionada con el sistema redox-glutation. Sin embargo, son necesarias nuevas investigaciones para confirmar estos hallazgos.

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Introduction

Community-acquired pneumonia (CAP) continues, even nowadays, to be one of the most common causes of admission in Intensive Care Units (ICU) with a significant morbidity. It is, therefore, not surprising that much research has been done in improving outcomes associated with this disease.

Reactive oxygen species (ROS) are constantly produced under physiological conditions and balanced between pro- and antioxidant activity. Nevertheless, in CAP, such activity might drastically be enhanced in response to primary host defence mechanism through phagocytes activation. Immune cell functions, activation of inflammatory cascades and expression of adhesion molecules are specially linked to ROS generation. Due to the respiratory burst generated by the massive flux of phagocytes, an important generation and release of ROS is produced.

The "respiratory burst" in a phagocyte is triggered when a bacteria is phagocytised. Therefore an important generation and release of ROS might perpetuate or increase the inflammatory response. Lipids, proteins and DNA damage oxidation leads to a tissue injury. In this context, the organism is overwhelmed by an imbalance between oxidant generation and antioxidant defenses; this situation is defined as oxidative stress (OS) and contributes to cellular derangement, cell injury and death.

Lipid peroxidation mediated by free radicals is considered to be the major mechanism of cell membrane destruction and cell damage. Some of the antioxidant systems that remove or inactivate ROS are superoxide dismutase (SOD; E.C. 1.15.1.1), catalase (CAT, E.C.1.11.1.6), glutathione reductase (GR), glutathione peroxidase (GPx, E.C. 1.11.1.9). Several studies have demonstrated that OS occurs in different settings affecting critically ill patients, such as in ARDS or organ dysfunction.

Only a few studies have focused on analyzing the role of OS in the pathophysiology of bacterial pneumonia in humans. Moreover, most of them are based on experimental models. In addition, little is known regarding the pathogenesis of 2009 H1N1 viral pulmonary infection and ROS production. In fact, viral and bacterial pulmonary infection may play a different role in the pro- and antioxidant balance in host cells. Thus, our aim was to compare the oxidative stress in severely ill patients with community-acquired pneumonia according to aetiology and with respect to a group of healthy volunteers and patients who suffered an acute myocardial infarction.

Materials and methods

Population samples

This study has been conducted according to the principles expressed in the Declaration of Helsinki and after obtaining local Ethics Committee approval. Signed informed consent was obtained from all patients or relatives and healthy volunteers. Oxidative stress data were not used in patients’ management and did not interfere with patient care.

Consecutive patients at ICU admission were included in a prospective and observational study with diagnosis of bacterial community-acquired pneumonia (BCAP) according to ATS/IDSA Guidelines or confirmed 2009 pandemic Influenza A/H1N1 pneumonia (VCAP) as reported elsewhere. In brief,
BCAP was defined as an acute lower respiratory tract infection characterized by (a) an acute pulmonary infiltrate evident on chest radiography and consistent with pneumonia; (b) confirmatory findings of a clinical examination; (c) acquisition of the infection outside a hospital, long-term care facility, or nursing home associated with positive bacteriologic data or with a favorable outcome following antibiotic therapy. An organism was considered to be a "definite" etiologic agent only if it could be isolated from samples of blood or pleural fluid or if serological tests revealed a four-fold increase in antibody levels. Isolation of *Pneumocystis jiroveci* or culture of *Legionella pneumophila* or *Mycobacterium tuberculosis* from any of the samples was considered to be the basis of a "definite" diagnosis. Other microorganisms isolated from sputum, tracheal aspirate, protected specimen brush (PSB), or bronchoalveolar lavage (BAL) fluid samples were considered to be "probable" pathogens. A urinary antigen test result positive for *Legionella pneumophila* or *Streptococcus pneumoniae* was considered to provide a "probable" etiology.

The 2009 A/H1N1 primary viral pneumonia (VCAP) was defined in patients presenting during the acute phase of influenza virus illness with acute respiratory disease and unequivocal alveolar opacification involving two or more lobes with negative respiratory and blood bacterial cultures. The 2009 A/H1N1 infection was confirmed by means of real-time reverse-transcription-polymerase chain reaction (RT-PCR) on either nasopharyngeal swab samples or tracheal secretions (bronchoaspirate). RT-PCR methods and further details are described elsewhere. A confirmed case was defined as an acute respiratory illness with laboratory-confirmed A/H1N1 and included in the study.

Severe CAP was defined as patients with CAP requiring ICU admission. Patients with HIV infection, neoplasia, those taking cytotoxic drugs or long-term oral steroid therapy, such as daily dose of 20 mg of prednisolone or the equivalent for >2 weeks were considered immune-compromised and were excluded.

Two groups were considered for comparison:

a) Control group (CG): Ten healthy volunteers were included; they were matched with patients according to age (±5 years).

b) Non-infectious group: Twelve patients with non-complicated acute myocardial infarction (AMI) were included. AMI was considered as a good group for comparison because it is an acute pathology of non-infectious origin, which induces a systemic inflammatory response with production of oxygen free radical and tissue damage. This non-infectious group of patients were matched by age (±5 years) and onset time of acute pathology (24 h) with the study group patients. AMI was defined as a clinical (or pathologic) event caused by myocardial ischemia in which there is evidence of myocardial injury or necrosis. Criteria are met when there is a rise and/or fall of cardiac biomarkers, along with supportive evidence in the form of typical symptoms, suggestive electrocardiographic changes, or imaging evidence of new loss of viable myocardium or new regional wall motion abnormality.

In all patients, clinical, demographics and biochemical variables were recorded to determine severity of illness by measuring Acute Physiology and Chronic Health Evaluation (APACHE II score), Sequential Organ Failure Assessment (SOFA score) and Systemic Inflammatory Response Syndrome (SIRS score).

### Groups analysis

Subjects were distributed into 3 groups: (1) CAP patients (study group), (2) healthy volunteers (control Group) and (3) AMI (non-infectious group) for comparison. Subsequently, we performed a comparison within the CAP group differentiating bacterial (BCAP) vs. 2009 A/H1N1 viral (VCAP) etiology. In addition, BCAP and VCAP patients were compared with healthy volunteers group.

### Samples collected

After obtaining informs consent from the patient or her/his legal representative and healthy volunteers, blood samples were obtained from all patients on ICU admission, day 3 and ICU discharge. In healthy volunteers only one blood sample was collected.

### Oxidative stress analysis

They were collected in lithium–heparin as well as EDTA tubes. The haemoglobin (Hb) concentration was determined by a spectrophotometer (Perkin Elmer Lambda 2) at 540 nm. The blood was centrifuged at 2000 rpm (850 × g) for 15 min to obtain plasmatic and erythrocytary fractions. Plasma was separated and stored at −80 °C until analysis. The erythrocytes were washed twice with saline solution, processed and stored at −80 °C until analysis. Index of oxidative injury (lipoperoxidative damage) was determined by MDA (Malondialdehyde). Antioxidant capacity was determined by copper–zinc superoxide dismutase (SOD), CAT and glutathione redox system (GSSG, GSH, GR, Gpx) activities. GSSG/GSH ratio was calculated as redox buffer index.

### Assay of plasma and erythrocytes MDA

MDA concentration was estimated by measurement of thio-barbituric acid reactant substances (TBARS) by a modified fluorescence method. Briefly, one volume of sample was mixed with 2 vol of a solution of 15% (w/v) trichloroacetic acid, 0.375% (w/v) thiobarbituric acid and 0.25 N hydrochloric acid, and the mixture heated for 15 min in a boiling water bath. After cooling, the precipitate was removed by centrifugation at 3000 rpm (1900 × g) for 10 min. Absorbance was measured at 548 nm. Plasma and erythrocytes MDA are expressed as nmol/ml and nmol/g Hb, respectively.

### Assays of enzymes activities

SOD activity in erythrocytes was measured by the epinephrine auto-oxidation according to the method described by Mishra et al. One unit of SOD activity was defined as the amount of enzyme required to inhibit the...
rate of epinephrine auto-oxidation by 50%. The SOD activity results are expressed in U/I/g Hb. CAT activity in erythrocytes was measured by the rate of decrease in the hydrogen peroxide (H₂O₂) absorbency at 240 nm. Results of CAT activity are expressed in mmol/min/g Hb. GPx activity in erythrocytes was determined by the decrease of NADPH at 340 nm as described Wheeler CR et al. The result is expressed as µmol/min/g Hb. GR activity was determined by the increase of NADP⁺ at 340 nm according to the method of Goldberg et al. GR activity is expressed in µmol/min/g Hb.

Assay of GSH and GSSG

GSH and GSSG concentrations were determined according to Hissin et al. The reduced and oxidized thiols were determined by reaction with O-phthalaldehyde for color development detected at 350 and 420 nm by a spectrophotometer Perkin Elmer LS 50B. Plasma levels are expressed as nmol/ml and erythrocytes levels are expressed as µmol/g Hb.

Statistical analysis

Discrete variables were expressed as counts (%) and continuous variables as mean and standard deviation (SD), unless stated otherwise; all statistical tests were two-sided. p < 0.05 was considered significant. Differences in categorical variables were calculated using two-sided likelihood ratio Chi-square test or Fisher exact test and the Mann–Whitney U test or Kruskal–Wallis test were used for continuous variables, when appropriate. To evaluate the predictive ability of the different biomarkers in regard with mortality, we calculated a receiver operator characteristic (ROC) curve and the area under the ROC (AUROC) curve. We calculate the AUROC because the area does not depend only on a particular portion of the plot, such as the point closest to the diagonal or the sensitivity at 90%, but also on the entire plot, and provides a comprehensive picture of the ability of a test to make a distinction over all decision thresholds.

Data analysis was performed using SPSS for Windows 13.0.0 (SPSS, Chicago, IL, US).

Results

Study group

Fifteen patients with severe CAP were included and 450 measurements of OS biomarkers were performed. Among the study group, 12 (80%) patients had bacterial CAP (BCAP) and 3 patients (20%) had 2009 A/H1N1 viral pneumonia without bacterial co-infection (VCAP). Clinical characteristics of patients are shown in Table 1. BCAP patients were older and had higher level of organic dysfunction (SOFA score) than patients with VCAP. However, APACHE II score, SIRS score, comorbidities and mortality were not different between groups.

Lipoperoxidative injury

At baseline, day-3 and ICU discharge patients with VCAP showed lower plasmatic TBARS levels than patients with BCAP. Erythrocyte TBARS levels were also lower in patients

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Table 1  Clinical characteristics of 15 patients with community-acquired pneumonia (CAP) differentiating bacterial (BCAP) and viral (VCAP) etiology.

<table>
<thead>
<tr>
<th>Variables</th>
<th>CAP overall (n = 15)</th>
<th>BCAP (n = 12)</th>
<th>VCAP (n = 3)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, median (SD) year</td>
<td>55.3 (19.3)</td>
<td>61.8 (14.8)</td>
<td>29.3 (10.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>12 (80.0)</td>
<td>10 (83.3)</td>
<td>2 (66.7)</td>
<td>0.51</td>
</tr>
<tr>
<td><strong>Severity of illness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>APACHE II, mean (SD)</td>
<td>16.0 (3.8)</td>
<td>16.3 (3.9)</td>
<td>14.7 (4.0)</td>
<td>0.53</td>
</tr>
<tr>
<td>SOFA score, mean (SD)</td>
<td>6.3 (3.0)</td>
<td>7.0 (2.7)</td>
<td>3.7 (2.7)</td>
<td>0.01</td>
</tr>
<tr>
<td>SIRS score, mean (SD)</td>
<td>2.9 (0.7)</td>
<td>3.0 (0.7)</td>
<td>2.7 (0.5)</td>
<td>0.50</td>
</tr>
<tr>
<td><strong>Comorbidities, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COPD</td>
<td>4 (26.6)</td>
<td>4 (33.3)</td>
<td>0 (0.0)</td>
<td>0.24</td>
</tr>
<tr>
<td>Asthma</td>
<td>2 (13.3)</td>
<td>1 (8.3)</td>
<td>1 (33.3)</td>
<td>0.25</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>4 (26.6)</td>
<td>4 (33.3)</td>
<td>0 (0.0)</td>
<td>0.24</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>2 (13.3)</td>
<td>2 (16.6)</td>
<td>0 (0.0)</td>
<td>0.44</td>
</tr>
<tr>
<td>Hypertension</td>
<td>4 (26.6)</td>
<td>4 (33.3)</td>
<td>0 (0.0)</td>
<td>0.25</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>5 (33.3)</td>
<td>5 (41.6)</td>
<td>0 (0.0)</td>
<td>0.17</td>
</tr>
<tr>
<td>Alcoholism</td>
<td>1 (6.6)</td>
<td>1 (8.3)</td>
<td>0 (0.0)</td>
<td>0.25</td>
</tr>
<tr>
<td><strong>Outcomes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mortality rate, n (%)</td>
<td>4 (26.6)</td>
<td>3 (25.0)</td>
<td>1 (33.3)</td>
<td>0.77</td>
</tr>
</tbody>
</table>

SD: standard deviation; n: number of patients; APACHE II: acute physiology and chronic health evaluation; SOFA: sequential organ failure assessment; SIRS: systemic inflammatory response syndrome; COPD: chronic obstructive pulmonary disease.
with VCAP during entire study period. However, this difference only achieved significance at ICU admission (Table 2).

**Antioxidant system capacity**

Throughout the study period, erythrocyte GR activity was significantly higher in patients with VCAP in respect of BCAP patients. No other significant differences were observed when different biomarkers of the enzymatic system were compared (Table 2). Within the biomarkers of non-enzymatic system, only plasmatic GSSG levels were significantly higher in patients with VCAP compared to BCAP patients during the entire study period (Table 2). No other significant differences were observed between groups, except for plasmatic GSSG/GSH ratio at ICU discharge.

**Microbiologic findings and oxidative stress**

Etiologic diagnosis was established in all cases of BCAP. A definite diagnosis was provided by blood culture in only 1 (8.3%) case. A probable diagnosis was provided by tests for urinary antigen detection in 4 cases (33.3%) and by bronchoscopic specimen or tracheal aspirate specimens with quantitative cultures in 8 (66.6%) patients. No diagnosis was made by sputum specimen culture. The most frequent microorganism isolated in BCAP was *Streptococcus pneumoniae* (*n=5*; 33.3%) followed by *Legionella pneumophila* (*n=3*; 20%), *Staphylococcus aureus* (*n=2*; 13.3%), *Enterococcus faecalis* (*n=1*; 6.7%) and *Proteus mirabilis* (*n=1*; 6.7%). No significant differences were observed when comparing oxidative stress biomarkers between pneumococcal and non-pneumococcal pneumonia. However, 2009 A/H1N1 viral aetiology was associated with lower levels of lipoperoxidation (Table 3).

**CAP group vs. healthy volunteers (control group)**

Ten healthy volunteers formed the control group. Plasmatic but not erythrocyte TBARS were higher in CAP patients. No differences were observed within enzymatic system between groups. Among non-enzymatic system, erythrocyte GSSG and GSSG/GSH ratio were higher in CAP patients. However, erythrocyte GSH and plasmatic GSSG were higher in HV (Table 4). CAP patients were older than control group patients due to the influence of patients with BCAP. This difference may explain the higher level of plasmatic TBARS observed. To avoid this possible confounding factor, we matched control group subjects with patients according to age. Two age-matched groups of healthy volunteers were compared with BCAP and VCAP patients respectively. Surprisingly, VCAP patients showed a lower level of lipoperoxidation with respect to controls. Significant differences were not observed when considering patients with BCAP (Table 4). Among enzymatic system, no significant differences were observed in all comparisons. However, GRE activity was higher in VCAP patients than control patients, although the difference was near to the significance (*p=0.08*). Lower levels of erythrocyte GSH and higher activity of GSSG E were observed in both BCAP and VCAP patients than in control group. In contrast, only BCAP patients showed lower levels of plasmatic GSSG than in control patients (Table 4).

**CAP group vs. non-infectious group (AMI patients)**

Twelve patients with acute myocardial infarction (AMI) were included in non-infectious patients group. Clinical characteristics of study and AMI group are shown in Table 5. Age, sex and comorbid condition were not different between

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**Table 2** Oxidative stress biomarkers at ICU admission (baseline), day 3 and ICU discharge in patients with community-acquired pneumonia (CAP) differentiating bacterial (BCAP) and viral (VCAP) etiology.

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Baseline</th>
<th>Day-3</th>
<th>ICU discharge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BCAP</td>
<td>VCAP</td>
<td>BCAP</td>
</tr>
<tr>
<td>Lipoperoxidation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBARS P (nmol/ml)</td>
<td>1.93 (0.8)*</td>
<td>0.64 (0.1)**</td>
<td>2.22 (1.0)*</td>
</tr>
<tr>
<td>TBARS E (nmol/gHb)</td>
<td>2.92 (1.4)*</td>
<td>0.93 (0.1)**</td>
<td>3.14 (1.9)</td>
</tr>
<tr>
<td>Enzymatic system</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOD E (U/gHb)</td>
<td>1639 (560)</td>
<td>1158 (81.7)</td>
<td>1845 (532)</td>
</tr>
<tr>
<td>CAT E (nmol/(min/gHb))</td>
<td>237.6 (54.7)</td>
<td>191.5 (40.6)</td>
<td>246.6 (34.5)</td>
</tr>
<tr>
<td>SOD/CAT</td>
<td>7.41 (3.8)</td>
<td>6.34 (1.8)</td>
<td>7.52 (1.8)</td>
</tr>
<tr>
<td>GR E (μmol/(min/gHb))</td>
<td>3.72 (2.0)</td>
<td>9.21 (7.6)**</td>
<td>3.01 (1.0)*</td>
</tr>
<tr>
<td>GPx E (μmol/(min/gHb))</td>
<td>29.01 (9.5)</td>
<td>33.92 (6.5)</td>
<td>28.32 (8.4)</td>
</tr>
<tr>
<td>Non-enzymatic system</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>GSH E (nmol/ml)</td>
<td>2.25 (1.3)</td>
<td>2.78 (0.3)</td>
<td>2.16 (1.5)</td>
</tr>
<tr>
<td>GSSG E (μmol/gHb)</td>
<td>1.36 (0.5)</td>
<td>1.49 (0.3)</td>
<td>1.13 (0.6)</td>
</tr>
<tr>
<td>GSSG/GSH E</td>
<td>1.24 (1.2)</td>
<td>0.53 (0.1)</td>
<td>1.33 (1.4)</td>
</tr>
<tr>
<td>GSH P (nmol/ml)</td>
<td>21.57 (10.1)</td>
<td>14.40 (3.3)</td>
<td>21.87 (11.0)</td>
</tr>
<tr>
<td>GSSG P (nmol/ml)</td>
<td>11.50 (4.3)*</td>
<td>26.63 (4.4)**</td>
<td>12.19 (5.8)*</td>
</tr>
<tr>
<td>GSSG/GSH P</td>
<td>0.89 (0.9)</td>
<td>1.88 (0.3)</td>
<td>0.92 (0.9)</td>
</tr>
</tbody>
</table>

* vs. **p < 0.05 for intergroup comparison.
groups. However, as expected, the severity of illness measured by the APACHE II, SOFA and SIRS scores was higher in patients with CAP.

Although patients with CAP had higher levels of plasmatic and erythrocyte TBARS than patients with AMI, this difference did not reach statistical significance in any of the moments of the study (Table 6).

At baseline and day-3, erythrocyte SOD activity and SOD/CAT ratio were lower in CAP patients than in AMI patients. No significant differences were observed in other biomarkers of enzymatic system analyzed in different moments of the study. Within the biomarkers of non-enzymatic system, only the plasmatic GSH levels at ICU admission and ICU discharge were significantly lower in patients with CAP than in AMI patients (Table 6).

### Mortality

ICU mortality of CAP was 26.6% (4/15). Three (25%) BCAP patients and 1 (33.3%) VCAP patient died. We found no significant differences in oxidative stress biomarkers between survivors and non-survivors at baseline and day-3 of study period (Table 7).

Discrimination for mortality of different biomarkers and severity of illness scores at baseline was assessed using ROC. The area AUROC showed no consistent mortality discrimination for plasmatic TBARS (AUROC = 0.32), GSH (AUROC = 0.42), GSSG (AUROC = 0.52), erythrocyte TBARS (AUROC = 0.44), GSH (AUROC = 0.58), GSSG (AUROC = 0.42), CAT E (AUROC = 0.52); GRE (AUROC = 0.36), GPX (AUROC = 0.44) and SOFA score (AUROC = 0.68). Only SOD E (AUROC = 0.72; 95% CI 0.46–0.98) and APACHE II score (AUROC = 0.90; 95% CI 0.40–1.0) showed adequate mortality discrimination.

### Discussion

The current pilot study is the first to compare temporal patterns of oxidative stress in bacterial and 2009 A/H1N1 viral CAP and their relationship with respect to healthy volunteers and non-infectious patients group. Our findings have shown an increased OS and high TBARS levels in bacterial CAP compared with healthy volunteers but not with patients suffering an AMI. However and surprisingly, our results suggest that 2009 A/H1N1 viral CAP led to an antioxidant environment evidenced by lower TBARS levels and increased some component of the glutathione redox system when compared with healthy subjects, bacterial CAP and non-infectious patients.

Although ROS are important in the successful resolution of certain inflammatory states, such as infections, an imbalance in the redox homeostasis can result in excessive tissue injury and death cell. During CAP, an early response of the immune system against the infection comprises the migration of neutrophils into the infected tissue. In vivo, S. aureus infection is associated with enhance nitrate generation, myeloperoxidase (MPO) activity, lipoperoxidation levels, Protein carbonil levels and decrease GSH levels, and as well as decreased enzymatic antioxidant (SOD, CAT, GPX and GRE) activity. The GS redox system is crucial in maintaining intracellular GSSG/GSH homeostasis, which is critical to normal cellular physiological processes, and represents one of the most important antioxidant defense systems in lung cells. In our study, CAP was associated with high levels of TBARS and a significant decrease of GSH levels when compared to healthy volunteers. This was accompanied with higher levels of GSSG and GSSG/GSH ratio. The maintenance of a low intracellular GSSG/GSH ratio minimizes accumulation of disulphide and provides a reducing environment within the cell. However, if any situation alters this ratio, this shift in the GSSG/GSH redox buffer influences a variety of cellular signaling processes, such as activation of the transcription factors AP-1 and NF-kB. Thus, depletion of intracellular GSH levels or increased GSSG levels is present concomitant with the induction of inflammatory mediators and chemotactic cytokines. This suggests that the intracellular redox state (GSSG/GSH levels) of the cell may play a role in the regulation and potentiation of the inflammatory responses in lung cells.
Oxidative stress in immunocompetent patients with severe community-acquired pneumonia

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Oxidative stress comparison between patients with community-acquired pneumonia (CAP) and control group of healthy volunteers in general and then matched by age.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
<td>CAP (n = 15)</td>
</tr>
<tr>
<td><strong>Biomarkers</strong></td>
<td></td>
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</tr>
<tr>
<td><strong>Enzymatic system</strong></td>
<td></td>
</tr>
<tr>
<td>SOD E (U/gHb)</td>
<td>1543 (536)</td>
</tr>
<tr>
<td>CAT E (nmol/(min/gHb))</td>
<td>228 (54.3)</td>
</tr>
<tr>
<td>GR E (µmol/(min/gHb))</td>
<td>4.8 (4.0)</td>
</tr>
<tr>
<td>GPx E (µmol/(min/gHb))</td>
<td>30.0 (9.0)</td>
</tr>
<tr>
<td><strong>Non-enzymatic system</strong></td>
<td></td>
</tr>
<tr>
<td>GSH E (nmol/ml)</td>
<td>2.3 (1.2)</td>
</tr>
<tr>
<td>GSSG E (µmol/(min/gHb))</td>
<td>1.3 (0.4)</td>
</tr>
<tr>
<td>GSSG/GSH E</td>
<td>1.14 (1.1)</td>
</tr>
<tr>
<td>GSH P (nmol/ml)</td>
<td>20.1 (9.5)</td>
</tr>
<tr>
<td>GSSG P (nmol/ml)</td>
<td>14.5 (7.5)</td>
</tr>
</tbody>
</table>
Myocardial ischemia or ischemia/reperfusion not only restores the blood supply but also causes massive production of free radicals, resulting in an imbalance between the oxidative and anti-oxidative balance and may initiate lipid peroxidation in cell membranes. This situation can explain why we did not observe differences in TBARS levels between CAP and AMI patients. Our findings are similar to those of the studies conducted by Dubois Rande et al.\textsuperscript{41} and Mac Murray et al.\textsuperscript{42} which reported a significant rise in TBARS levels with a concomitant decrease in antioxidant in patients with unstable angina and chronic heart failure.

Despite the limited sample size, it important to stress that the TBARS levels were lower in the VCAP with respect to the BCAP, AMI and healthy volunteers respectively. The impact of OS in BCAP has been extensively proved, however the role of blood antioxidants according to CAP etiology has not been totally elucidated. Our results suggest that an antioxidant status might play an important role in patients...
with 2009 A/H1N1 viral pneumonia. GR, GSSG, GSSG/GSH and GPx levels were more elevated in patients with viral pneumonia. Experimental data\(^a\) suggest that influenza virus infection increases gene expression of antioxidants in the lungs. Influenza virus may have a direct effect on lung antioxidants gene expression and does not require the presence of innate immune response.\(^b\) According to our findings, in VCAP patients, the antioxidant status could be related with an increased activity of the redox glutathione system to a greater extent than SOD and CAT enzymes. An experimental study in mice\(^c\) observed that despite an increased expression of SOD mRNA, there was no increase in SOD activity in the lung homogenates from virus-infected animals. This antioxidant status could lead to a minor damage of biologic membrane and explain why VCAP patients had less organ failure when compared to those with BCAP.

The present study has several potential limitations that should be addressed. First, the number of patients included is limited and therefore, our results should be interpreted cautiously and further confirmed in a larger size of the population. In order to minimize a bias based on the number of patients included, patients with BCAP and VCAP were compared to a control group that comprised healthy volunteers and other non-infectious group of patients. In addition, age might be a confusion factor and to avoid a bias, control group was age-matched into two groups and compared to BCAP and VCAP patients. Second, this is an observational, not interventional study in which only adults with severe disease were included. In addition, only patients with viral infection due to 2009 A/H1N1 strain were studied, therefore our results might not be generalized to other populations with others virus strain and mild forms. Third, we did not measure other oxidant/antioxidant substances. However, oxidative stress can be investigated either by detection of oxygen free radicals and other similar substances (ROS), or by detection of antioxidants and damage products of essential biomolecules (e.g. lipid peroxidation products) as a consequence of ROS activity. Our research was performed according to the second investigation line.

In conclusion, our preliminary data might suggest the occurrence of pro-oxidant/anti-oxidant imbalance in CAP patients with respect to healthy volunteers. In contrast, a higher antioxidant activity in patients with 2009 A/H1N1 viral pneumonia was observed. Changes in antioxidant activity might be associated with a lower organ dysfunction according to the SOFA score. A better knowledge of the molecular mechanisms that sequentially regulate this battery of genes in relation to GSH levels in lung cells may open new therapeutic avenues in the modulation of inflammatory responses in lung diseases.

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### Conflict of interest

The authors have no conflicts of interest to disclose.

### References