



Outcome Predictors of Severe Acute Exacerbation of Chronic Obstructive Pulmonary Disease: Role of Inflammatory Biomarkers

Hanaa Shafiek^{1*}, Nashwa Abd-elwahab¹, Manal Baddour², Akram Degady³, Mohamed El-hoffy¹ and Yehia Khalil¹

¹Chest Diseases Department, Faculty of Medicine, Alexandria University, Egypt

²Microbiology and Immunology Department, Faculty of Medicine, Alexandria University, Egypt

³Clinical and Chemical Pathology Department, Faculty of Medicine, Alexandria University, Egypt

***Corresponding author:** Hanaa Shafiek, MD, PhD, Chest Diseases Department, Faculty of Medicine, Alexandria University, El-Khartoom Square, Alexandria 21526, Egypt, Tel: 002-01095907320, E-mail: whitecoat_med@yahoo.com

Abstract

Objective: To assess the value of interleukin-6 (IL-6), interleukin-8 (IL-8) and C-reactive protein (CRP) in predicting the outcome of acute respiratory failure (ARF) on top of COPD.

Patients and methods: Serum samples were collected from 33 COPD patients presented with ARF for IL-6, IL-8, and CRP analysis on admission, after 72 hours and after 14 days. Sputum samples were taken for microbiological evaluation.

Results: During the study, 75.8% survived and 24.2% died. Serum IL-6 only on admission was significantly higher ($p = 0.03$) among the non-survivors [376.0 (interquartile range (IQR)= 26.3-511) pg/ml] vs. the survivors [8.2 (IQR = 0.1-17)pg/ml] as well as after 14 days ($p = 0.04$). Both the CRP and IL-8 were higher among the non-survivors without significant difference ($p > 0.05$). The IL-6 after 72 hours showed statistical significance ($p = 0.03$) in predicting the outcome being lower among those discharged on room air [0.1 (IQR = 0.1-0.3)pg/ml] compared to either the non-survivors or those discharged on new supplemental oxygen therapy or continuous positive airway pressure [1.6 (IQR = 0.1-187.6) and 7.2 (IQR = 0.1-41.3)pg/ml respectively]. IL-6 > 46.1 pg/ml on admission had the sensitivity of 71% and specificity of 84% for predicting in-hospital mortality ($p = 0.042$); but CRP > 2.3 mg/L had the best sensitivity (85.7%). Gram-negative bacteria- the commonest pathogen among non-survivors- was insignificantly associated to the outcome ($p = 0.262$).

Conclusions: High IL-6 is associated with in-hospital mortality in ARF on top of COPD. Both CRP and IL-6 when used together, they become good in-hospital mortality predictors.

Keywords

Acute respiratory failure, Interleukin-6, In-hospital mortality, C-reactive protein, Gram-negative bacteria

Introduction

Acute exacerbations of chronic obstructive pulmonary diseases (AECOPD) are an important problem for healthcare systems, because of the morbidity and mortality rates. These episodes are associated with accentuation of both airway and systemic inflammation [1]

besides the worsening in airway function and respiratory symptoms in patients with chronic obstructive pulmonary diseases (COPD) [2] AECOPD can range from self-limited episodes to acute respiratory failure (ARF) requiring mechanical ventilation [3]. A range of potential factors have been studied both as risk factors of exacerbations resulting in hospitalization as well as predictors of mortality and other outcomes [4]. These factors include forced expiratory volume in one second (FEV₁), partial pressure of arterial oxygen (PaO₂), partial pressure of arterial carbon dioxide(PaCO₂), oxygen saturation and resting oxygen uptake, low body mass index (BMI), current smoking status, older age, low serum albumin, the comorbidities, severity of illness and functional status [4].

Various markers were reported to be higher in blood during exacerbation compared with the baseline include C-reactive protein (CRP), interleukin 8 (IL-8), tumor necrosis factor- α (TNF- α), leptin, endothelin-1, eosinophil cationic protein, myeloperoxidase, fibrinogen, interleukin 6 (IL-6), α 1-antitrypsin, and leukotriene E4, leukotriene B4 and copeptin [5]. The aim of the present study was to assess the value of some inflammatory biomarkers (IL-6, IL-8 and CRP) in predicting the outcome of ARF on top of COPD, to propose cutoff value of the studied biomarkers for predicting in-hospital mortality and assessing its sensitivity and specificity. Part of this study have been published [6].

Methods

Study design and population

A cross sectional prospective pilot study enrolled 33 patients with COPD diagnosisas defined by GOLD [7] presented with acute respiratory failure attending the respiratory intensive care unit (RICU) of the chest diseases department, Alexandria Main University Hospital, Alexandria, Egypt between June 2010 and August 2011. All the patients were followed up for 30 days. The study was approved by local Ethical Committee.

Characteristics of the patients

All patients at recruitment were in acute respiratory failure as defined by arterial blood gas (ABG) criteria (PaO₂ < 60 mmHg, with

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or without $\text{PaCO}_2 > 45 \text{ mmHg}/\text{pH} < 7.35$) during breathing room air [8] with the presence of acute respiratory distress. Further, all the patients were not admitted to the hospital in the last 30 days before recruitment. Any patient suffering from other confounding inflammatory diseases, such as malignancy, arthritis, connective tissue disorders or inflammatory bowel disease as well as all patients with other significant respiratory diseases including asthma, tuberculosis and primary bronchiectasis were excluded from the study.

All the patients on admission were subjected to thorough history taking (including age, gender, smoking history, exacerbations/year, and comorbidities). Baseline dyspnea was evaluated using "The Modified Medical Research Council (MMRC) dyspnea scale scoring" [9] full clinical examination including weight and height, laboratory investigations (mainly complete blood picture, serum albumin, serum electrolytes, creatinine, blood urea nitrogen), plain chest X-ray and ABG.

After initial evaluation, the patients were managed according to the international guidelines [10]. The patients were assigned to the standard drug protocol, supplemental oxygen therapy plus NIV as initial trial if not contraindicated and maintained as long as it is tolerated. The patients were monitored during the NIV for oxygen saturation, clinical state and hemodynamic state. ABG was further ordered if the patient did not improve or showing clinical evidence of deterioration. Failure of NIV was defined as termination of NIV trial and initiation of invasive mechanical ventilation (MV).

Microbiological evaluation

Sputum samples or bronchial wash –performed for selected cases– were obtained on admission from all patients for microbiological analysis using both ordinary cultures for common pathological bacteria and polymerase chain reaction (PCR) technique for atypical pathogens (*Mycoplasma* and *Chlamydia*). Details of the bacteriological detection were previously publication [6]. Briefly, an equal volume of the sputum was mixed with sterile saline and incubated at room temperature for 15 minutes with intermittent shaking for homogenization of sputum. For the bronchial wash, no dilution was done. Samples were inoculated on Blood agar and MacConkey's agar for quantification of pathogens. Identification of the infecting pathogens was carried out according to standard microbiological procedures including Gram staining and biochemical reactions followed by antibiotic susceptibility testing. Polymerase chain reaction (PCR) was done on the collected sputum or bronchial lavage sample for detection of *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*. DNA was extracted from all samples using the GeneJET™ Genomic DNA Purification Kit (Fermentas, Thermo Scientific) followed by amplification of DNA sequence which was carried out in a Techne Progene thermal cycler. Electrophoresis was carried out at 80 volts for 25 minutes in order to detect the amplified products. DNA fragments (280 bp for *Mycoplasma pneumoniae* and 474 bp for *Chlamydia pneumoniae*) were visualized by ethidium bromide staining against an ultraviolet transilluminator [11].

Serum samples and analysis of the inflammatory markers

Serum samples were obtained (on admission, after 72 hours and on the 14th day) and were preserved in -80°C till the end of the study period. The collected samples were analyzed for IL-6, IL-8 and CRP. The IL-6 and IL-8 were measured in serum samples by ELISAKit (AviBion human IL-6 and IL-8, code number IL06001 and IL08001 respectively, Finland) according to the manufacturers' directions. CRP was measured in serum samples by CRP-ultrasensitive (MICRO CRP/ ULTRA CRP, Vital Diagnostics, Italy). This kit utilizes quantitative turbidimetric latex technique.

Statistical analysis

Quantitative data are presented as means \pm standard deviation (SD) while categorical variables as frequencies and percentages (%) unless otherwise stated. All statistical tests including Kruskal-Wallis test, Mann-Whitney test, Monte-Carlo test and Spearman rank

Table 1: Demographic and clinical characteristics of recruited patients.

| Baseline Characteristics | Cases (N = 33) |
|--|-------------------------------|
| Age (years) | 56.61 \pm 7.87 |
| Gender | |
| Male/Female | 26 (79%) / 7 (21%) |
| Smoking status | |
| Current smokers / Ex-smokers / Passive smokers | 11 (33%) / 15 (46%) / 7 (21%) |
| Smoking index [p/yr (mean \pm SD)] | 40.25 \pm 22.96 |
| Type of smoking | |
| Cigarette smoking | 24 (72.7%) |
| Water pipe smoking | 2 (6%) |
| Presence of co-morbidities | |
| Hypertension | 13 (37%) |
| Diabetes Mellitus | 7 (21%) |
| OSA | 10 (30.3%) |
| Ischemic heart disease | 3 (9.1%) |
| Others | 3 (9.1%) |
| Pulmonary hypertension; mPAP (mmHg) | 23 (88.5%); 31.35 \pm 6.45 |
| Pulmonary function test: | |
| FEV ₁ / FVC | 53.41 \pm 10.45 |
| FEV ₁ (L) | 1.23 \pm 0.58 |
| FEV ₁ % predicted | 43.7 \pm 18.2 |
| History of exacerbation | |
| < 3 times/year; previous MN | 11 (33%); 3 (9%) |
| ≥ 3 times/year; previous MV | 22 (67%); 6 (18%) |

OSA: obstructive sleep apnea, p/yr: pack/year, mPAP: mean pulmonary artery pressure, FEV₁: forced expiratory volume in 1st second, FEV₁/FVC ratio: the forced expiratory volume in 1st second / the forced vital capacity.

correlations were employed as appropriate unless otherwise stated. Post Hoc testing was used for multiple comparisons corrections. Receiver Operating Characteristic (ROC) curve analysis and area under the curve (AUC) were used to identify the best cut-off points of each of IL-6, CRP, and IL-8 and calculate its sensitivity and specificity in predicting in-hospital mortality. Cox regression hazard and Kaplan Meier analysis were applied to study different parameters dependency of survival. Both the hazard ratio and 95% confidence interval (CI) were shown. All applied statistical tests of significance were two-sided and $p \leq 0.05$ was considered as statistically significant. Statistical analysis was carried out using Microsoft excel and Statistical Package for Social Sciences (SPSS version 11, Chicago, IL, USA).

Results

Patients' characteristics

Baseline clinical and physiological characteristics of the 33 patients are shown in [table 1](#). The majority of our patients were severe to very severe COPD with average FEV₁ % predicted of 43.7 \pm 18.2%. Twenty-five patients (75.8%) survived and 8 patients (24.2%) died during the study; where 2 patients only of non-survivors (6%) were alive on the 14th day and died on days 17th and 20th of the study. Those who survived were discharged from the hospital either on room air [14 patients (43%)] or new supplemental oxygen therapy/CPAP [11 patients (33%)]. The causes of mortality were either new insult i.e., hospital acquired infection and tension pneumothorax, the persistence of initial infection with progression to shock and a baseline of very severe COPD. The characteristics of the two groups (survivors and non-survivors) are shown in [table 2](#).

There was a statistically significant difference between the survivors and non-survivors regarding the BMI index (29.8 \pm 7.8 versus 21.6 \pm 4.2 Kg/m^2) and dyspnea grade (2.4 \pm 0.9 versus 3.6 \pm 0.5) ($p = 0.008$ and 0.001 respectively; [table 2](#)). In addition, there was a statistically significant difference between the chloride level of the survivors (96.6 \pm 3.6 mmol/L) and that of the non-survivors (92 \pm

Table 2: Comparison between the survivors and non-survivors regarding different clinical, physiological, radiological and laboratory variables

| Variable | Survivors (n = 25) | Non-survivors (n = 8) | p value |
|--|-----------------------|--------------------------|-------------------------|
| Age (years) | 55.2 ± 6.9 | 61.1 ± 9.4 | 0.091 |
| Smoking History | | | |
| Current smoker | 8 (32%) | 2 (25%) | ^{FE} p = 1.0 |
| Smoking index | 38.7 ± 25.4 | 46.3 ± 11.9 | 0.13 |
| BMI (kg/m ²) | 29.8 ± 7.8 | 21.6 ± 4.2 | 0.008* |
| Dyspnea (MMRC scale) | 2.4 ± 0.9 | 3.6 ± 0.5 | 0.001* |
| Comorbidities | 18 (72%) | 6 (75%) | ^{FE} p = 1.0 |
| mPAP | 31 ± 6.1 | 32.1 ± 12 | 0.643 |
| Exacerbation ≥ 3 times/yr; Y/N | 16/9 (64%) | 6/2 (75%) | ^{FE} p = 0.687 |
| Failure of NIV trial; Y/N (%) | 4/21 (16%) | 4/4 (50%) | ^{FE} p = 0.074 |
| Plain chest X-ray [n (%)] | | | |
| Consolidative patch ^a , | 11 (44%) | 4 (50%) | ^{FE} p = 1.0 |
| Consolidative patch 14 th day | 1 (4%) | 2 (25%) [§] | ^{FE} p = 0.01* |
| Laboratory investigations | | | |
| hematocrit (%) | 49.8 ± 7.5 | 43.8 ± 7.6 | 0.101 |
| Total WBC (10 ³ /µL) | 10 ± 4.6 | 17.3 ± 10.5 | 0.065 |
| Na (mmol / L) | 138.8 ± 4.4 | 133.3 ± 8.7 | 0.08 |
| K (mmol / L) | 4.3 ± 0.6 | 4.2 ± 0.6 | 0.583 |
| Ca (mg/dl) | 8.3 ± 0.5 | 8.2 ± 0.5 | 0.844 |
| Cl (mmol / L) | 96.6 ± 3.6 | 92 ± 6.6 | 0.034* |
| BUN (mg/dl) | 25.9 ± 16.5 | 32.6 ± 12.5 | 0.077 |
| Cr (mg/dl) | 0.9 ± 0.5 | 1.2 ± 0.7 | 0.33 |
| albumin (g/dl) | 3.0 ± 0.5 | 2.8 ± 0.4 | 0.19 |
| ABG on admission | | | |
| pH | 7.31 ± 0.05 | 7.34 ± 0.10 | 0.385 |
| PCO ₂ (mmHg) | 70.5 ± 12.6 | 61.5 ± 32.0 | 0.264 |
| PO ₂ (mmHg) | 48.1 ± 13.8 | 61.5 ± 32.0 | 0.569 |

BMI (kg/m²): body mass index (kilogram/meter²), WBC: white blood count, Na: sodium, Ca: calcium, K: potassium, Cl: chloride, BUN: blood urea nitrogen, Cr: creatinine, Y/N: yes/no, yr: year, a: admission, ABG: arterial blood gases, PaO₂ (mmHg): partial arterial pressure of oxygen, PaCO₂ (mmHg): partial arterial pressure of carbon dioxide, [§]: developed new consolidation on the 14th day, significance by Mann Whitney test, ^{FE}p=: Fisher's Exact test, *significant if p ≤ 0.05.

6.6 mmol/L (p = 0.034; **table 2**). However, there was no significant difference between the two groups regarding the age, exacerbation history, smoking status and index, ABG, failure of NIV trial, blood urea nitrogen, creatinine, the remaining electrolytes, serum albumin, the hematocrit and total white blood count as shown in **table 2**.

Regarding the plain chest X-ray taken on admission there was no statistically significant difference between the survivors and non-survivors concerning the presence of consolidative patches (p = 1.0); however, on the 14th day 25% of the non-survivors acquired new patch indicating hospital acquired infection which gave a statistically significant difference when compared to survivors (4% only had non-resolving patch) (p = 0.01; **table 2**).

Microbiologically

Causative microorganism was detected in 66% of the sputum samples. Gram-negative bacteria were detected in 9 sputum samples (31%). Atypical bacteria were identified in 8 patients (28%) in the form of *Mycoplasma* (25%) mainly; more details regarding microorganism cultured could be reviewed in our previously publication [6]. Gram-negative bacterial infection was the most frequent (66.7%) among the non-survivors. The atypical bacterial infection was the most frequent (36.4%) among the patients discharged on O₂ therapy and/or CPAP, but undetected bacteria were more prevalent (41.7%) among those discharged on room air. However, there was no statistically significant association between the type of infection and the outcome of the patients on the 14th day (p = 0.262).

Inflammatory biomarkers

IL-6 showed a statistically significant difference between the

Table 3: Comparison between levels of studied biomarkers (IL-6, IL-8 and CRP) at different stages of the study according to survival of patients[§]

| Biomarkers | Stages | Survival | | P value |
|-----------------|----------------------|-----------------------------|--------------------------------|--------------------------|
| | | Survivor (n = 25; 75.8%) | Non-survivor (n = 8; 24.2%) | |
| IL-6 (pg/ml) | Admission | 8.2 (0.1-17) | 376.0 (26.3-511) | <i>z</i> = 2.186 (0.03)* |
| | 72 hours | 0.1 (0.1-7.2) | 1.6 (0.1-187.6) | <i>z</i> = 0.855 (0.393) |
| | 14 th day | 3.1 (0.1-23.2) | 76.3 (68.5-84.1) | <i>z</i> = 2.05 (0.04)* |
| IL-8 (pg/ml) | Admission | 15 (9.9-28.9) | 29.2 (9.1-164.5) | <i>z</i> = 0.661 (0.508) |
| | 72 hours | 18.3 (11.2-53.5) | 13.9 (4.8-23.6) | <i>z</i> = 0.95 (0.342) |
| | 14 th day | 29.4 (12.8-155.8) | 97 (61.7-132.4) | <i>z</i> = 0.501 (0.616) |
| CRP (mg/L) | Admission | 3 (1.8-3.4) | 2.4 (2.4-3.3) | <i>z</i> = 0.498 (0.618) |
| | 72 hours | 2.1 (0.4-2.7) | 1.8 (0.7-2.3) | <i>z</i> = 0.15 (0.881) |
| | 14 th day | 2.2 (0.6-3.5) | 3.1 (3-3.1) | <i>z</i> = 0.602 (0.547) |

IL-6: interleukin 6, IL-8: interleukin 8, CRP: C-reactive protein, *z*: Mann Whitney test, [§]all data are presented in median and interquartile range (IQR), *significant at p ≤ 0.05.

Table 4: Comparison between the levels of studied inflammatory biomarkers on admission and after 72 hours according to the outcome on the 14th day[§]

| Biomarkers | Stages | Outcome on the 14 th day | | | p value | |
|-----------------|-----------|-------------------------------------|--------------------------|---|---|--|
| | | Non-survivors (8; 24.2%) | Survivors (25; 75.8%) | | | |
| | | | On room air (14; 43%) | On O ₂ therapy or CPAP (11; 33%) | | |
| IL-6 (pg/ml) | Admission | 376.0 (26.3-511) | 9.8 (0.1-39.4) | 6.8 (0.3-14.2) | <i>x</i> ² = 4.846 <i>p</i> = 0.089 | |
| | 72 hours | 1.6 (0.1-187.6) | 0.1 (0.1-0.3) | 7.2 (0.1-41.3) | <i>x</i> ² = 7.003 <i>p</i> = 0.03* | |
| IL-8 (pg/ml) | Admission | 29.2 (9.1-164.5) | 14.4 (10.4-26.1) | 16.8 (9.6-28.9) | <i>x</i> ² = 0.447 <i>p</i> = 0.799 | |
| | 72 hours | 13.9 (4.8-23.6) | 12.7 (7.9-24.8) | 28.2 (13.0-55.6) | <i>x</i> ² = 3.125 <i>p</i> = 0.2 | |
| CRP (mg/L) | Admission | 2.4 (2.5-3.3) | 2.4 (2.0-3.4) | 3.0 (1.9-3.5) | <i>x</i> ² = 0.844 <i>p</i> = 0.656 | |
| | 72 hours | 1.8 (0.7-2.3) | 1.4 (0.2-2.2) | 2.5 (2.0-2.8) | <i>x</i> ² = 4.687 <i>p</i> = 0.096 | |

IL-6: interleukin-6, IL-8: interleukin-8, CRP: C-reactive protein, O₂: oxygen, CPAP: continuous positive airway pressure, *x*²: Kruskal Wallis test, [§] all data are presented in median and interquartile range (IQR), *significant at p ≤ 0.05.

survivors and non-survivors on admission 8.2 (interquartile range (IQR) = 0.1-17) vs. 376.0 (IQR = 26.3-511) pg/ml respectively; *p* = 0.03, **table 3**). Similarly, on the 14th day there was a statistically significant difference between the survivor group 3.1 (IQR = 0.1-23.2) vs. non-survivor group (2 patients) 76.3 (IQR = 68.5-84.1) pg/ml; *p* = 0.04, **table 3**).

Further, IL-6 level after 72 hours has statistically significant difference in predicting the outcome on the 14th day (*p* = 0.03, **table 4**) as those discharged on room air had the lowest value (0.1 (IQR = 0.1-0.3) pg/ml) compared to either the non-survivors or those discharged on oxygen therapy or CPAP (1.6 (IQR = 0.1-187.6) and 7.2 (IQR = 0.1-41.3) pg/ml respectively). However, neither the CRP nor the IL-8 showed any significant difference when comparing the survivors and non-survivors or in predicting the outcome (*p* > 0.05; **table 3** and **table 4**). Further, the inflammatory biomarkers did not show regression among the survivors over the study duration despite the apparently initial regression in IL-6 values after 72 hours (**figure 1**).

Correlations

There was no statistically significant correlation between the studied inflammatory markers' level on admission and the following: BMI, dyspnea grade, the exacerbation history, the smoking status and the associated co-morbidities (*p* > 0.05). However, a statistically significant positive correlation was found between the IL-6 and the total white blood count and inverse correlation with the hematocrit value (*r* = 0.423, *p* = 0.014; *r* = -0.411, *p* = 0.017 respectively; **figure 1**).

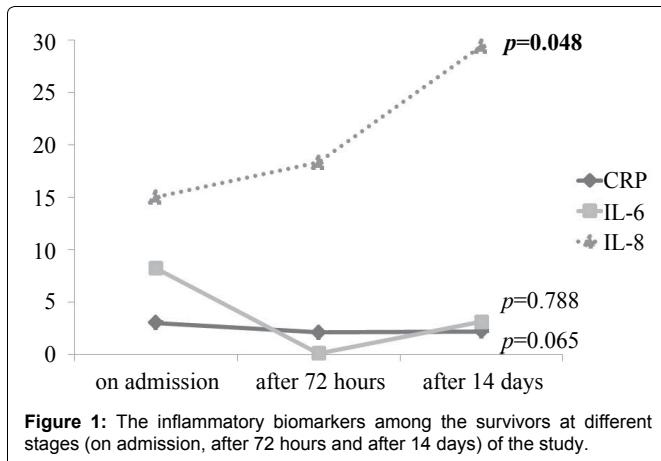


Figure 1: The inflammatory biomarkers among the survivors at different stages (on admission, after 72 hours and after 14 days) of the study.

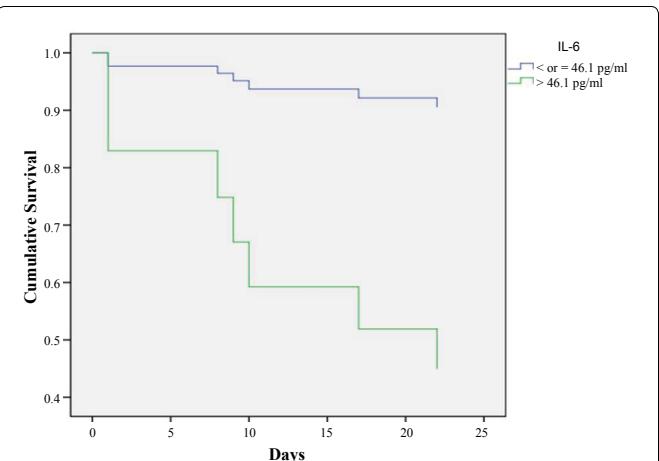


Figure 4: Kaplan Meier analysis for IL-6 value on admission.

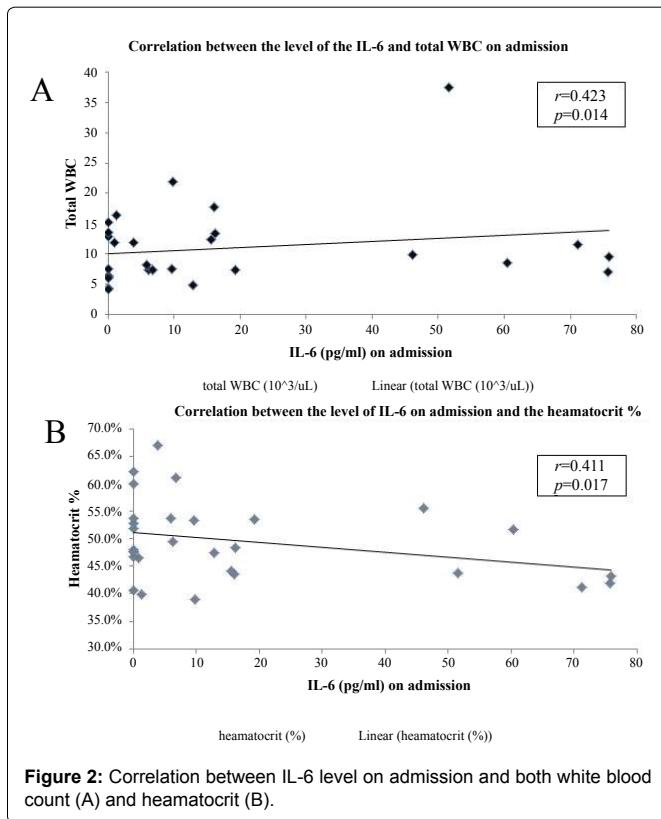


Figure 2: Correlation between IL-6 level on admission and both white blood count (A) and hematocrit (B).

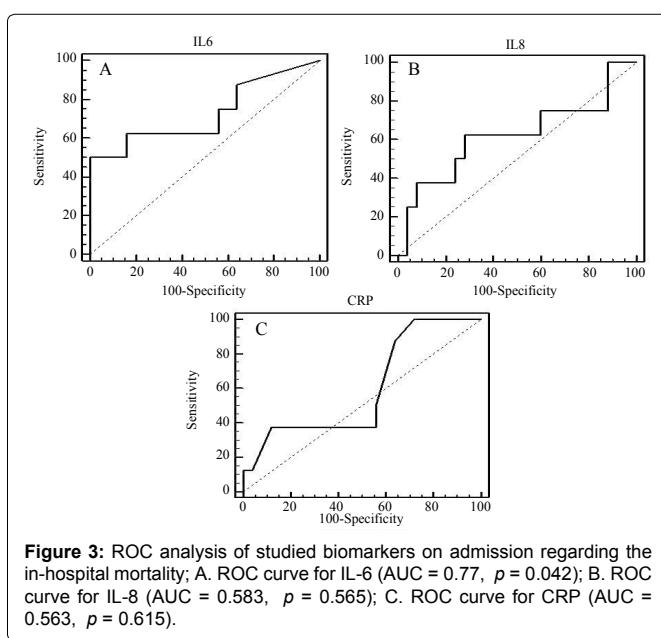


Figure 3: ROC analysis of studied biomarkers on admission regarding the in-hospital mortality; A. ROC curve for IL-6 (AUC = 0.77, $p = 0.042$); B. ROC curve for IL-8 (AUC = 0.583, $p = 0.565$); C. ROC curve for CRP (AUC = 0.563, $p = 0.615$).

2). Moreover, there was a statistically significant positive correlation between CRP and IL-6 ($r = 0.298, p = 0.005$). In addition, there was a statistically significant positive correlation between the CRP and the smoking index ($r = 0.514, p = 0.007$).

ROC and regression analysis

The cutoff point of IL-6 is > 46.1 pg/ml which shows a sensitivity of 71% for predicting “in-hospital mortality” and a specificity of 83% (AUC = 0.77, $p = 0.042$; figure 3-A). The cutoff point of IL-8 is > 28.6 pg/ml which has a lower sensitivity (57%) and specificity (72%) (AUC = 0.583, $p = 0.565$; figure 3-B). Regarding CRP, the cutoff point is > 2.3 mg/L which has high sensitivity (85.7%) for predicting mortality but the lowest specificity (37.5%) (AUC = 0.563, $p = 0.615$; figure 3-C).

Moreover, Cox proportional hazards regression analysis showed that only IL-6 value on admission was strongly associated to the probability of in-hospital mortality with a hazard ratio of 2.5 (CI 95% = 1.2-5.01; $p = 0.013$) with 60% probability of survival at 14 days in case of value > 46.1 pg/ml (figure 4); while the value of the same biomarker at 14 days had a hazard ratio of 3.4 (CI 95% = 0.7-16.3) but it did not reach statistical significance ($p = 0.132$). None of the other biomarkers or the ABG parameters showed significant hazard ratio in relation to in-hospital mortality.

Discussion

In the current study we showed accentuated systemic inflammation in non-survivors presented with severe AECOPD especially regarding IL-6. Further, IL-6 level on admission was the only biomarker with high specificity for predicting in-hospital mortality during AECOPD associated with ARF rather than CRP and IL-8 despite the high sensitivity of CRP.

Previous studies

Seneff *et al.* [12] and Ai-Ping *et al.* [13] showed that mortality was not related to baseline functional capacity, comorbidities including the presence of cor-pulmonale, ABG, previous hospitalization, or the use of invasive MV. Also, Aburto *et al.* [14] found no significant relationship between the admission ABG, serum albumin or hematocrit levels and the in-hospital mortality rate; and Potgieter *et al.* [15] found that the occurrence of nosocomial pneumonia is associated with an increased risk of fatalities. Additionally, Ong *et al.* [16] and McGhan *et al.* [17] showed in their studies that dyspnea score and weight loss respectively can predict the in-hospital mortality among admitted AECOPD patients being linked to increased systemic inflammation as expressed by elevated CRP, TNF- α or IL-6 [18,19]. Our results agreed with these observations. Further, the overall in-hospital mortality in our RICU was 24.2% which is within the range of previous studies [12,20].

Interpretation of the main results

We found that all the studied inflammatory biomarkers were

higher among the non-survivors rather than the survivors with a statistically significant difference regarding IL-6; and that IL-6 value > 46.1 pg/ml on admission showed the highest specificity (83%) in predicting in-hospital mortality and was associated with reduced survival to 60% at 14 days. Interestingly, IL-6 value after 72 hours was significantly higher among the non-survivors and the survivorpatients discharged on O₂ therapy or CPAP and the least value was among those discharged on room air. These findings raise the potential of the strong prognostic value of IL-6 and could reflect severe underlying lung disease. This could be explained on the basis that activated epithelial cells and increased numbers of alveolar macrophages and other inflammatory cells in COPD may release IL-6 into the circulation [21] which in turn increased during AECOPD [1,22]. Various mechanisms demonstrated the role IL-6 in the pathogenesis of COPD. Firstly, IL-6 increases the number of lung CD4 cells, CD8 cells, B cells, neutrophils, and macrophages [23,24] consistent with the changes observed in human COPD pathology [25]. Secondly, overexpression of IL-6 leads to emphysema-like airspace enlargement, peribronchiolar collections of mononuclear cells, thickening of airway walls, subepithelial fibrosis, and airway hyperresponsiveness [23,26]. Lastly, lung injury is attenuated by the absence of IL-6 after exposing animals to ozone [27].

Furthermore, there was a statistically significant positive correlation between the IL-6 and total WBC which supports the concept of increased systemic inflammation associated with AECOPD [1,22,28]. Also, IL-6 inversely correlated with the haematocrit in the present results. It has been proven that the increased levels of inflammatory cytokines lead to a shortened RBC survival, hence predispose to anemia [29]. Markoulakiet al. [30] found that during admission for AECOPD, hemoglobin levels are decreased and erythropoietin hormone levels are increased. This association is mainly related to increase IL-6 levels, indicating a possible erythropoietin resistance through the mechanism of increased systemic inflammatory process [30]. Additionally, IL-6 correlated positively with CRP; this is due to the fact that the CRP is primarily produced by hepatocytes in response to IL-6 stimulation [31].

We found that the chloride level was significantly lower among the non-survivors compared to the survivors. Mc Mahon *et al.* [32] who analyzed the data of 51,789 critically ill patients found that hypochloremia proved to be an independent predictor of mortality of all-cause mortality among critically ill patients, even after adjustment for sodium, although the mechanisms for this association remained unclear. However, hypochloremia is caused by multiple factors including: a) extrarenal causes as inadequate NaCl intake, losses of gastrointestinal fluids (e.g. vomiting, nasogastric suction); b) renal causes (e.g. diuretic abuse); c) conditions associated with adrenal insufficiency (e.g. lack of endogenous or exogenous glucocorticoids or mineralocorticoids), d) dilutional causes as early stages of hyperglycemia, syndrome of inappropriate antidiuretic hormone, decreased effective circulatory blood volume as in edema states), e) acid-base abnormalities (e.g. compensated respiratory acidosis); all ofthese causes are commonly associated with the critically ill patients [33].

According to the current results, we observed that the inflammatory biomarkers did not regress significantly during recovery among the survivors especially for IL-8 (Figure 2). However, IL-6 showed insignificant regression after 72 hours which could be due to therapeutic agents as corticosteroids [34] and antibiotics that modulate the inflammatory cascade [35]. Some studies [36-38] previously reported insignificant improvement of the biomarkers during recovery from AECOPD up to 2 months [38] denoting persistent systemic inflammation post-exacerbation. Kersul *et al.* [39] also observed similarly continue high levels of inflammation after resolution of exacerbation episode especially regarding IL-8. This could be explained by the molecular mechanism that regulates the transcription of inflammatory genes in the cell nucleus which is basally reduced in COPD [40] does not increase during treatment of the exacerbation with glucocorticoids.

Finally, neither the CRP nor the IL-8 showed good specificity for predicting in-hospital mortality despite the highly detected sensitivity of CRP (87.5%). This could be explained by the fact that CRP by itself is not specific in case of AECOPD [41] and could be affected by many factors as inflammatory response elsewhere in the body, infection, antibiotic treatment and use of inhaled corticosteroids [42] as well as smoking index [43] that positively correlated with CRP in the current study.

Clinical implications

Our pilot study demonstrates the role ofIL-6 in severe AECOPD and its relation to fatal outcome among this group of patients. Accordingly, we suppose that incorporation of IL-6 in the early evaluation of AECOPD could help better identification of the severe episodes especially of being inexpensive and easy test. Secondly, directed therapyagainst IL-6 or its receptor could improve the outcome of AECOPD by decreasing the burden of systemic inflammation in COPD patients which could be the nidus for frequent AECOPD [44] especially if infection is not the cause. Directed therapy against inflammation has been raised during the last decade [45].

Limitations of the study

The present study has some limitations. Firstly, we studied only the systemic inflammation and we did not evaluation the extent of the airway inflammation during severe AECOPD. However, some studies previously showed that during the exacerbation of COPD, the systemic inflammation reflects in an acceptable way the airway inflammation [1]. Secondly, we did not evaluate the validity of IL-6 or the other studied biomarkers (IL-8 and CRP) as long-term prognostic factors for patients presented with severe AECOPD as our study was for short term.

Conclusion

High serum levels of IL-6, lower BMI, higher dyspnea grade, low serum chloride, newly developed consolidative patch in the plain chest X-ray on follow up were predictors of bad prognostic outcome and high fatality rate. Further, IL-6 (> 46.1 pg/ml on admission) could be considered as single biomarker with good sensitivity and specificity for predicting in-hospital mortality. Also, CRP showed high sensitivity in predicting in-hospital mortality despite being non-specific. Accordingly, both of CRP and IL-6 when used together, they become good mortality predictors.

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Disclosure

The authors declare that there is no conflict of interest regarding the publication of this paper.

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