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Prediction of respiratory complications guided by Clara cell protein CC16 in plasma in polytrauma patients

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Abstract

***Objective*: To evaluate the value of serum levels of Clara cell protein (CC16) as a diagnostic and prognostic utility in patients with polytrauma and relate these levels to respiratory complications compared to plasma levels of healthy control group.**

***Subjects and methods*: A prospective cohort study was carried out on one hundred and fifty patients with polytrauma (blast, blunt, and penetrating) who admitted to Intensive Care Units of Maadi and Kobry El Kobba Hospitals, Cairo, Egypt, from June 2016 and June 2019. Full history taking, clinical examination, radiology investigations, laboratory investigations, CC16 protein and other inflammatory biomarkers were investigated.**

***Results*: There was no statistically significant difference between both groups as regarding age (p=0.09), comorbid conditions (p>0.05), Glasgow coma scale (p=0.09), Acute Physiology and Chronic Health Evaluation (APACHE) II score (p=0.07) and abbreviated injury scale (p=0.08). Along 5 days there was a significantly higher C-reactive protein (CRP) level, neutrophil-lymphocyte ratio in Group I. PO2 level was significantly higher in the first 2 days in Group II than Group I. Chest computed tomography (CT) scan revealed the presence of lung contusion in 30 patients, hemothorax in 20 patients, and pneumothorax in 25 patients, all in Group I. Also, there was a statistically significant difference on day 0 between both groups with much higher serum CC16 in Group I than Group II, while on day 3 the difference was insignificant. Whereas, respiratory complications were significantly higher serum and broncho alveolar lavage (BAL) level of CC16 on day 3, while this difference was insignificant on day 0.**

***Conclusions*: These findings showed that we may benefit from detecting serum CC16 levels in polytrauma victims in prediction of respiratory complications.**

Key words: Acute lung injury, Clara cell protein 16, inflammatory biomarkers, polytrauma, respiratory complications.

Introduction

Despite extensive research and increasing awareness of acute lung injury (ALI) and acute respiratory distress syndrome (ARDS), the diagnosis remains clinically challenging and may be missed in 50% of patients. (1) With growing evidence that specific treatments, including low-tidal volume ventilation and conservative fluid management, improve outcomes in ALI/ARDS. (2) There is a clear need for improved diagnostic approaches. Clara cell secretory protein, also known as CC16, is a secreted product of the respiratory epithelium that is produced primarily within the Clara cells of the distal respiratory and terminal bronchioles. (3) The biological function of CC16 remains incompletely understood, although CC16 has been demonstrated to interact with multiple components of the inflammatory and coagulation cascades. CC16 inhibits phospholipase A2 activity in vitro and in vivo, (4) suggesting it plays a role in attenuating inflammatory responses. CC16 has also been implicated in feedback inhibition of interferon gamma signalling, as well as modulation of T

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helper 2 responses to proinflammatory stimuli. (5)
Furthermore, CC16 appears to be activated by tissue transglutaminases including activated Factor XIII, and inhibits thrombin-stimulated platelet aggregation, suggesting a possible role in modulating the dysregulated coagulation characteristic of ALI/ARDS. (6) CC16 has been investigated as a potential biomarker of lung epithelial injury in numerous disease states including idiopathic pulmonary fibrosis, sarcoidosis, chronic obstructive pulmonary disease (COPD), asthma, occupational or environmental lung injury, bronchiolitis obliterans, chronic tobacco use, and ALI/ARDS. (7)
Several studies have examined whether CC16 levels in broncho alveolar lavage (BAL) fluid or plasma can discriminate patients with ALI/ARDS from those at-risk for ALI/ARDS or healthy control subjects. Nevertheless, the results to date have been contradictory. (8)
CC16 has recently gained acceptance as a blood biomarker for detecting direct and indirect lung injury. Although the early elevation of CC16 serum levels has been shown to correlate with pulmonary damage in patients with multiple injuries, the subsequent time course of CC16 serum levels has not been investigated in these patients. (9)
The purpose of this study is to evaluate the value of serum levels of CC16 as a diagnostic and prognostic utility in patients with polytrauma and relate these levels to respiratory complications compared to plasma levels of healthy control group.

Subjects and methods

A prospective cohort study was carried out on one hundred and fifty patients with polytrauma (blast, blunt, and penetrating) who admitted to Intensive Care Units of Maadi and Kobry El Kobba Hospitals, Cairo, Egypt, during June 2016 and June 2019.

Ethical consideration

The study was approved by the Ethical Committee of Faculty of Medicine, Cairo University and an informed consent obtained from all patients before the study was commenced.

Selection criteria for the patients

The subjects included in this study were selected according to inclusion and exclusion criteria.

Inclusion criteria

All trauma patients older than 18 years admitted to the Emergency Room who were transferred to the Intensive Care Unit (ICU) after initial treatment due to their life-threatening condition.

Exclusion criteria

Decompensated heart failure, age below 18 years, and previous hospital admission for 14 days before study.

Variables that were measured

- Full history (if possible): Age and sex, diabetes mellitus, hypertension, cardiac diseases, drug abuse (alcohol).
- Hours since injury: To determine the time since injury to hospital.
- Cause of injury: A strong association between the cause of injury and long-term outcome in moderate to severe trauma patient was observed.

Full clinical examinations

- Admission (systolic blood pressure [SBP], diastolic blood pressure [DBP], and mean arterial blood pressure [MAP]): Blood pressure was measured manually by sphygmomanometer. MAP was calculated as: MAP=(SBP+2xDBP)/3. We considered MAP<70 mmhg was hypotension. Cerebral perfusion pressure (CPP) was calculated as: CPP=MAP-intra cranial pressure (ICP).
- Admission heart rate (HR): HR=beats per min (bpm) (heart rate recorded from monitor or by counting pulse rate). We considered HR>100 bpm was tachycardia. (10)
- Admission respiratory rate (RR): Recorded respiratory rate from monitor or by counting cycle to detect breathing and rhythms. We considered RR>20/min was tachypnea. (10)
- Temperature: By applying the thermometer to patient axilla as oral temperature cannot be measured in disturbed conscious level and in the presence of maxilla-facial trauma. Temperature >38.5 °C was considered as fever. Seventy-nine percent (85/108) of TBI patients had at least one recorded fever event while in the ICU. The mean maximal temperature of this cohort of critically ill TBI patients was 39.0 °C (range 37.3-41.8 °C). (10)
- Pupil reactivity: We use direct light (torch) to detect if two eyes reactive, one eye reactive, or both non-reactive to evaluate the severity of TBI as non-reactive pupil(s) is an early indicator of increased ICP due to progression of the hematoma/hemorrhage or cerebral edema and this is an indirect method to get an idea about ICP. Pupil reaction abnormalities are: 1) Dilated and fixed, 2) Anisocoria: unequal pupil reactions, 3) Doll's eye response: eyes move

with head during rotation, and 4) Presence of nystagmus. (11)

- Convulsion: We asked emergency medical services (EMS) members and/or observed all patients whether they developed convulsions or not that worsened brain metabolism, elevate ICP, and rhabdomyolysis.
- Major extracranial injury (MEI): by examining of spine, thorax, abdomen, pelvis, back, extremities, external (e.g. contusions, burns, or abrasions). MEI is an important prognostic factor for mortality in TBI patients.
- Radiology investigations:
  - X-ray: Plain X-ray to head, spine, chest (to detect pneumothorax and/or hemopneumothorax), pelvis, and limbs to detect breaks or fractures of the skull or other bones in the body.
  - Brain CT: Brain CT was done for all head injury patients: severe head injury (Glasgow Coma Score [GCS] less than or equal to 8), moderate head injury (GCS 9-12), and mild head injury (GCS 13-14).
- Electrocardiography (ECG): ECG evaluation is not routinely helpful in the initial evaluation and treatment of the patient with severe polytrauma. Though there are no studies which show that it improves outcomes, cardiac monitoring is generally recommended.
- Pelvi-abdominal ultrasound: This performed at the bedside and is the investigation of choice in hemodynamically unstable patients. Free fluid in a hemodynamically unstable patient indicates the need for emergency laparotomy.
- Echocardiography: Transthoracic echocardiography was done for the detection of traumatic cardiovascular injuries in patients suffering from severe TBI and blunt chest trauma. All patients underwent transthoracic echocardiography within 8 hours of admission.

Laboratory investigation

- Arterial blood gases (ABG) (including pH, PaO2, and PaCO2) was done by radial artery puncture and the sample was sent immediately for analysis. ABG provides important information (hypoxia PaO2<60 mmHg, hypercapnia PaCO2>45 mmHg, acidosis pH<7.35) in major trauma victims.
- Complete blood count (CBC) (including Hb, hematocrit, white blood cells (WBCs), and platelets) was done for all trauma patients. Consideration: Hb<10 g/dl or hematocrit <30% as anemia, WBCs>11,000/mm^3 as leukocytosis, and platelets<150,000/mm^3 as thrombocytopenia. Normal hemoglobin and hematocrit results did not rule out significant hemorrhage. We did not withhold blood transfusion in patients who had relatively normal hematocrit results, but had evidence of clinical shock, serious injuries, e.g. open-book pelvic fracture, or significant ongoing blood loss. We used platelet transfusions to treat patients with thrombocytopenia and ongoing hemorrhage.
- Coagulation profile, including prothrombin time (PT), activated partial thromboplastin time (aPTT), international normalized ratio (INR), and platelet counts, are important to exclude a coagulopathy. A limited trauma-induced coagulopathy as evidence by prolonged PT and INR>1.5 levels have been found in patients hospitalized for traumatic injuries.
- Blood electrolytes (Na and K): Sodium disorders are the most common and most poorly understood electrolyte disorders in neurosurgical patient, hyponatremia (Na<125 mmol/litre) is association with mortality in trauma patients. K<3.5 mmol/l considered as hypokalemia in patients with head trauma upon admission to the ICU. These electrolyte imbalance occurred within hours of the trauma and resolved under treatment within the first day.
- Random blood sugar (RBS): Severe head injury is associated with a stress response that includes hyperglycemia (RBS>200 mg/dl), which worsens outcome before or during cerebral ischemia. To better define the relationship between human head injury and hyperglycemia, glucose levels were measured in 100 consecutive brain-injured patients from hospital admission.
- Renal function was done and we considered serum creatinine >1.5 mg/dl as renal impairment. We obtained renal function tests and creatinine kinase levels to detect rhabdomyolysis if a crush injury had occurred, or marked convulsion and rigidity were present.
- Serum lactate level: The normal blood lactate concentration in unstressed patients is 0.5-1 mmol/l. Patients with critical trauma illness can be considered to have normal lactate concentrations of less than 2 mmol/l.
- Total creatinine phospho-kinase (CPK): When total CPK level is very high, it usually means there has been injury or stress to muscle tissue, the heart, or the brain. CPK>260 ng/ml was considered as index of kidney injury, and pre-

caution against myoglobinuria should be started, such as good rehydration (CVP>12 cm H2O) and diuretics.

Specific laboratory investigations

- CC16 protein: Healthy controls were assessed once for plasma level of CC16 protein. Patients were assessed for plasma samples of CC16, which was obtained from left over samples (9 ml in endotoxin-free heparinized syringe) for routine clinical care of the patient at time of admission and 2 days after. Samples were centrifuged 2000 gravitational force (g-force) for 15 minutes at 4 °C), and the plasma was removed and stored at -80 °C until assayed. CC16 was measured in duplicate by a sandwich enzyme-linked immunosorbent assay previously validated in plasma and BAL fluid matrices (ELISA; polyclonal anti-human CC16 protein antibody; entire procedure, 4 hours).
- Sputum culture: Sputum culture was assessed for bacterial and fungal growth on day 0 and day 2-3 routinely after endotracheal tube suction sample withdrawal.
- Other inflammatory biomarkers: Quantitative C-reactive protein (CRP) level, total leucocyte count (TLC), differential TLC, and neutrophil/leucocyte ratio were assessed daily till the 5th day of ICU stay.

Lung mechanics

Measurements of static, dynamic lung compliance, peak, and plateau pressure with different ventilation settings was recorded in conjunction with ABG parameters.

All cases were evaluated on admission according to GCS, the Acute Physiological and Chronic Health Evaluation (APACHE) II scoring system, and Abbreviated Injury Score (AIS).

Primary outcomes (most important outcomes to be assessed): measure was difference in concentration of CC16 in plasma between subjects of polytrauma.

- High lightening cut off value for prognostic level of serum CC16 protein in prediction of outcome for trauma-related ARDS.
- Correlation between serum CC16 level and CRP, neutrophil-lymphocyte ratio among patients with polytrauma who did not develop vs who developed respiratory complications.

Statistical analysis

Results were tabulated and statistically analyzed by using a personal computer using Microsoft Excel 2016 and SPSS v. 21 (SPSS Inc., Chicago, IL, USA). Statistical analysis was done using descriptive (e.g. percentage [%], mean and standard deviation) and analytical (that included Mann-Whitney U test, chi-squared (χ^2), and receiver operating characteristic [ROC] curve). A value of p less than 0.05 was considered statistically significant.

Results

The current study showed that mean age of the studied groups was 38.16±11.9 years ranged from 20 to 60 years. There was no statistically significant difference between both groups as regarding age (p=0.09), comorbid conditions (p>0.05), Glasgow Coma Scale (p=0.09), APACHE II score (p=0.07), and Abbreviated Injury Scale (p=0.08) (**Table 1**).

Comparison between the two groups as regard to vital signs (arterial blood pressure, heart rate, temperature, and respiratory rate) were monitored for five days, and insignificant variation was found in the majority of cases (**Table 2**).

Also, there was statistically significant differences between the studied groups regarding solid organ affection, long bone fractures, respiratory complications, hemodynamic support, need for tracheostomy, and duration of mechanical ventilation. Meanwhile, length of ICU stay and Murray score were not significantly different among the studied groups. Fifteen patients in I had liver injury versus 2 patients in Group II, only one patient in Group II had spleen injury, and another one had both liver and spleen injury. Sixty-five patients in Group I had long bone fractures versus 50 patients in Group II. As post traumatic respiratory complications, we noticed that 30 patients developed respiratory complications (25 patients in Group I and 5 patients in Group II). Hemodynamic support was needed in 26 patients in Group I and 5 patients in Group II. Tracheostomy was done only for 14 patients out of 150 patients in Group I. Moreover, patients in Group I were intubated and mechanically ventilated with a mean duration of 3.8±5.92 versus 5 patients in Group II with a mean duration of 1±0.25 (**Table 3**).

In the current study, along the 5 days there was a significant higher CRP level, neutrophil-lymphocyte ratio in Group I (**Table 4**).

Blood gas data in both groups showed that PO2 level was significantly higher in the first 2 days in Group II than Group I, but in the latter 3 days there was insignificant difference. Meanwhile, pH, PCO2, HCO3, lactate, and oxygen saturation showed insignificant difference between both groups (**Table 5**).

Regarding sputum cultures, on day 0 there were 12 positive results in Group I and only 5 positive results in Group II. Meanwhile, on day 3 there were 26 positive results in Group I and only 5 positive results in Group II. Statistically there were significant differences with p value 0.03 and 0.01, respectively (**Table 6**).

In **Table 7**, chest CT scan revealed the presence of lung contusion in 30 patients, hemothorax in 20 patients, and pneumothorax in 25 patients, all in Group I. There was also a statistically significant difference on day 0 between both groups with much higher serum CC16 in Group I than Group II, while on day 3 the difference was insignificant. Whereas, in respiratory complications there were significantly higher serum and BAL level of CC16 on day 3 though this difference was insignificant on day 0.

Table 8 shows that serum CC16 level (13.7 ng/ml) on day 3 was related to high incidence for occurrence of respiratory complications with specificity 90%, sensitivity 86%, and p value 0.0001. The correlation between CC16 and other markers (CRP, neutrophil-lymphocyte ratio, lactate) was presented as a comparison between its significance in prediction of respiratory complications in polytrauma patients in our study. Plasma CC16 and CC16 BAL on day 3 was found to have the highest significant area under the curve with p value 0.0001 (Figure 1).

At last, **Table 9** revealed that 30 out of 150 patients died in hospital. Its mortality was higher in Group I than in Group II.

Discussion

Trauma is considered to be one of the main causes of death in the world and the number of deaths caused by traumatic incidents have experienced an annual increase of 15%. Delayed pulmonary complications are more common among traumatic patients. (12) Patients with thoracic trauma are presumed to be at higher risk for pulmonary dysfunction, and ARDS may develop in any patient, regardless of associated chest injury. (13)

Despite advancements in the initial resuscitation, critical care, and ventilator management, the development of ARDS is still a challenging problem for the intensivist. (14) Use of biomarkers capable of predicting potentially preventable adverse outcomes in patients with multiple traumas continues to be an interesting topic of research. Biomarkers capable of identifying trauma victims at risk for pulmonary complications would be of great help in clinical practice because their levels could be obtained early and objectively and are not subject to personal interpretation. (15)

The purpose of this study was to evaluate the value of serum levels of CC16 as a diagnostic and prognostic utility in patients with polytrauma and relate these levels to respiratory complications compared to plasma levels of healthy individuals. Polytrauma patients in our study were defined as all trauma patients older than 18 years who were transferred to the ICU after initial treatment due to their life-threatening condition. Burn victims and patients with a known history of malignancies, inflammatory diseases or other lung disorders were excluded, in order to provide comparative values.

In our study plasma samples of CC16 were obtained at time of admission then other samples were obtained later from BAL and plasma on day 3 using a commercially available ELISA kit. There was a statistically significant difference on day 0 between both groups with much higher serum CC16 in Group I (patients who had direct thoracic lesion) than Group II (patients who did not have direct thoracic lesion) while on day 3 the difference was insignificant.

The comparison between both who developed respiratory complications (ARDS or acute lung injury) with those who did not develop respiratory complications revealed significantly higher serum and BAL levels of CC16 on day 3 in those who developed respiratory complications.

In concordance to our study, Jorens PG, et al (16) found that CC16 levels were elevated in both the serum and BALF among ARDS patients, and higher levels were associated with increased mortality.

Prospective study was conducted by Sanjeev Kumar, et al (17) to assess the serum level of CC16 and volume of the uninvolved lung by Multi-Detector Computed Tomography (MDCT) and to ascertain the correlation between levels of CC16 with the volume of the injured lung in the clinical outcome of patients of blunt thoracic trauma. Higher level (more than 100 ng/ml) of serum Clara cell protein was associated with the requirement of ventilator and mortality, and can give us an idea about the extent of lung damage by thoracic trauma besides providing a cheap and easily available rapid test for determining future clinical course of the patients.

Similarly, in trauma-associated ARDS, Wutzler S, et al (18) found that the serum levels of CC16 were significantly higher in the trauma patients who developed severe lung injury compared with non-ARDS patients and healthy individuals. The CC16 levels were also found to be correlated with the

volume of the lung contusions. Wutzler S, et al (19) in a diagnostic study confirmed the previously described association between initial elevation in CC16 serum levels and severe thoracic injury in patients with multiple injuries, the investigators collected an extensive series of blood samples from the time the patients were admitted until 14 days after trauma. They found that the initial CC16 levels were significantly elevated, but if no secondary respiratory complications occurred, the levels declined to the control values within the first day after trauma. These results suggest that elevated CC16 is a specific biomarker for traumatic ARDS, and they mentioned that the timing of sampling seems to be an important factor that might influence the CC16 concentrations. Lukas, et al (20) in a prospective observational study found that levels of CC16 in polytraumatized patients differed remarkably from those of a healthy control group and CC16 levels assessed on day 2 (24 to 48 hours after trauma) was found to be most appropriate to predict pneumonia in polytraumatized patients with severe chest trauma. Moreover, CC16 levels were lower in survivors than in non-survivors. Zhao, et al. (21) conducted a study to examine the clinical value of continuously monitoring serum CC16 levels in diagnosing pulmonary contusion, estimating its severity degree and predicting the disease progression. They found that the maximum volume of lung contusion had a positive correlation to the initial and average concentrations of CC16, and that CC16 may serve as a biomarker to assist clinical diagnosis and monitor the progression of pulmonary contusion, which may provide a simple and effective reference basis for clinical treatment decisions.

A pilot study was conducted by Suresh Kumar, et al (22) aiming to assess the role of von Willebrand factor (vWf) and CC16 as a prognostic marker in patients with isolated, blunt, or penetrating traumatic lung injury. Serum levels were estimated in venous samples by ELISA method at the time of admission. They found that serum levels were correlated with severity of lung injury, duration of hospital stay, and final outcomes.

But Phillip Stormann, et al (23) in a retrospective observational multicenter study concluded that no reliable predictive or surveillance biomarkers including CC16 could be established for clinical diagnosis and identification of patients at high risk for acute traumatic lung injury. Nevertheless, there are plenty of promising markers that need to be further elucidated in larger case numbers and multicenter studies.

One of the interesting findings of our study was that a positive relationship with strong association between serum levels of CC16 (day 1) in ventilated patients and Murray score. It supports the Clara cell protein as a new marker that may be beneficial in the prediction of respiratory complications among trauma patients especially those who may be candidates for extracorporeal membrane oxygenation (ECMO). We hypothesized that early prediction of respiratory complications among polytrauma patients will help us in taking the clinical decisions, setting the plane of treatment and making therapeutic maneuvers such as proceeding for early tracheostomy, early consultation of a pulmonologist or transportation to the nearest ECMO center as well as dealing with the expectations of the patient's relatives as it is considered a very important issue in our culture.

Conclusion

These findings showed that we may benefit from detecting serum CC16 levels in polytrauma victims in prediction of respiratory complications.

Recommendations

Obtaining plasma CC16 and CC16 from BAL on day 3 using a commercially available ELISA kit. Also, obtaining serum CC16 levels on admission in trauma victims as it may have a diagnostic value in detecting patients with severe chest injuries.

Table 1. Comparison between the studied groups regarding age, comorbid condition, GCS, APACHE II, and Abbreviated Injury Scale

		Studied groups		p value
		Group I (n=75)	Group II (n=75)	
Age (year)	Mean±SD Range	38.16±9.2 20-60	39.72±11.9 20-60	0.9
Comorbid conditions	Smoking	62/75	75/75	0.07
	DM	13/75	14/75	0.06
	HTN	15/75	13/75	0.06
	CNS lessions	0/75	0/75	0.06
	Cardiac insult	0/75	0/75	0.06
GCS		9.48±2.8	10.46±2	0.9
APACHE II		14.60±7.8	12.77±5.6	0.07
Abbreviated Injury Scale		5±1	4±1	0.8

Legend: GCS=Glasgow Coma Score; APACHE II=Acute Physiological and Chronic Health Evaluation; DM=diabetes mellitus; HTN=hypertension; CNS=central nervous system.

Table 2. Comparison between the studied groups regarding vital signs

Vital signs		Studied groups		p value
	Day	Group I (n=75)	Group II (n=75)	
Blood pressure (mmHg)	1	82.14±12.3	90.41±11.5	0.58
	2	78.2±8.7	94.52±11.6	0.69
	3	84±11.5	92±11.4	0.44
	4	87.98±11.6	94.73±11.6	0.33
	5	89.15±11.3	90.21±11.6	0.64
Heart rate (beats/min)	1	99.44±9.3	100.53±2.4	0.9
	2	88.01±15.9	99.83±4.7	0.91
	3	84.58±17.4	96.34±6.9	0.61
	4	82.14±18.5	83.18±9.7	0.92
	5	84.09±20.1	88.97±9.5	0.31
Temperature (°C)	1	37.32±0.4	38.42±0.19	0.92
	2	37.15±0.40	37.8±0.5	0.9
	3	37.19±0.3	37.65±0.7	0.9
	4	37.33±0.4	37.85±0.6	0.98
	5	37.85±0.7	38.16±0.6	0.99
Respiratory rate (breaths/min)	1	24.54±4.7	20.73±1.3	0.43
	2	23.56±5.5	21.57±1.6	0.78
	3	23.97±6.6	20.54±2.5	0.76
	4	23.88±6.3	19.77±2.5	0.56
	5	22.55±6.3	18.98±3.2	0.53

Table 3. Comparison between the studied groups regarding solid organ, long bone fractures, respiratory complications, hemodynamic support, tracheostomy, mechanical ventilation, ICU stay, and Murray score

		Group I	Group II	Total	p value
Solid organ (n)	Liver	15	2	17	0.01
	Spleen	0	1	1	0.1
	Both liver and spleen	0	1	1	0.1
Long bone fractures (n, %)		65 (86.6%)	50 (66.6%)	115 (76.6%)	0.04
Respiratory complications (n)		25	5	30	0.001
Need hemodynamic support (n)		26	5	31	0.001
Need for tracheostomy (n, %)		14 (18.6%)	0 (0.0%)	14 (18.6%)	0.01
Need for mechanical ventilation Duration of mechanical ventilation (mean±SD)		25 3.8±5.92	5 1±0.25	30	0.01
Length of ICU stay (mean±SD)		8.04±0.42	9.37±0.15		0.09
Murray score (mean±SD)		3.5±0.7	2.5±0.5		0.07

Legend: ICU=intensive care unit.

Table 4. Comparison between the studied groups regarding CRP and neutrophil-lymphocyte ratio

	Day	Group I (n=75)	Group II (n=75)	p value
C-reactive protein (mean±SD)	1	15.09±8.25	9.89±4.33	0.05
	2	23.54±10.78	12.76±5.77	0.04
	3	51.54±10.56	17.56±7.55	0.03
	4	51.76±11.98	20.54±10.24	0.03
	5	56.87±21.21	24.74±12.89	0.03
Neutrophil-lymphocyte ratio (mean±SD)	1	78.89±2.58	38.98±2.87	0.02
	2	79.78±4.89	46.09±5.67	0.03
	3	78.98±4.09	58.98±5.98	0.03
	4	78.98±4.09	38.98±4.32	0.01
	5	89.93±4.08	75.09±3.09	0.04

Legend: CRP=C-reactive protein.

Table 5. Comparison between the studied groups regarding arterial blood gases data

	Day	Group I (n=75)	Group II (n=75)	p value
pH (mean±SD)	1	7.37±0.05	7.35±0.02	0.89
	2	7.37±0.05	7.36±0.01	0.89
	3	7.37±0.06	7.35±0.04	0.89
	4	7.37±0.07	7.36±0.04	0.89
	5	7.34±0.09	7.35±0.04	0.89
PO2 (mean±SD)	1	66.53±24.45	116.99±13.58	0.03
	2	67.48±23.42	115.63±15.17	0.04
	3	86.20±22.28	98.46±11.53	0.68
	4	88.82±20.17	97.81±8.67	0.77
	5	82.62±15.71	87.46±10.97	0.87
CO2 (mean±SD)	1	35.86±5.43	36.65±3.18	0.28
	2	35.86±4.50	43.86±2.35	0.54
	3	36.44±4.67	43.20±1.97	0.59
	4	38.30±6.35	39.10±2.26	0.91
	5	40.42±8.44	43.62±3.22	0.86
HCO3 (mean±SD)	1	22.68±2.96	20.23±3.05	0.76
	2	22.81±2.93	19.44±3.05	0.66
	3	36.44±4.67	43.20±1.97	0.91
	4	38.30±6.35	39.19±2.36	0.96
	5	40.42±8.44	43.62±3.22	0.85
Lactate (mean±SD)	1	2.87±1.03	3.75±0.77	0.98
	2	3.03±1.65	4.15±0.98	0.87
	3	3.28±2.07	4.27±1.16	0.86
	4	3.16±1.92	3.99±1.56	0.91
	5	3.53±2.81	3.79±2.11	0.97
Oxygen saturation (mean±SD)	1	93.82±3.72	91.33±1.46	0.88
	2	93.42±3.36	92.90±1.36	0.85
	3	93.75±3.75	93.91±1.68	0.99
	4	92.88±3.59	92.75±1.27	0.99
	5	91.35±5.04	91.85±1.42	0.99

Table 6. Comparison between the studied groups regarding culture results on day 0 and 1

Culture results		Group I	Group II	Total	p value
Day 0	Positive (n)	12	5	17	0.03
	Negative (n)	63	70	133	
Day 1	Positive (n)	26	5	31	0.01
	Negative (n)	49	70	119	

Table 7. Comparison between the studied groups regarding CT, CC16 and respiratory complications

		Group I	Group II	Total	p value
CT	Normal	0	75	75	NA
	Contusion	30	0	30	NA
	Hemothorax	20	0	20	NA
	Pneumothorax	25	0	25	NA
CC16 (mean±SD)	CC1	11.6640±0.4	7.2520±0.3	-	0.04
	CC3	8.6453±2.6	6.9200±2.4	-	0.07
	BALCC3	9.5893±3.6	7.2307±2.8	-	0.07
Respiratory complications					
CC1	No	50	70	120	0.1
	Yes	25	5	30	
CC3	No	50	70	120	0.02
	Yes	25	5	30	
BALCC3	No	50	70	120	0.01
	Yes	25	5	30	

Legend: CT=computed tomography; CC16=Clara cell secretory protein; CC1=Clara cell secretory protein on day 1; CC3=Clara cell secretory protein on day 3; BALCC3=bronchoalveolar lavage Clara cell secretory protein on day 3.

Table 8. Cut off value of CC16 on day 3 for prediction of respiratory complications

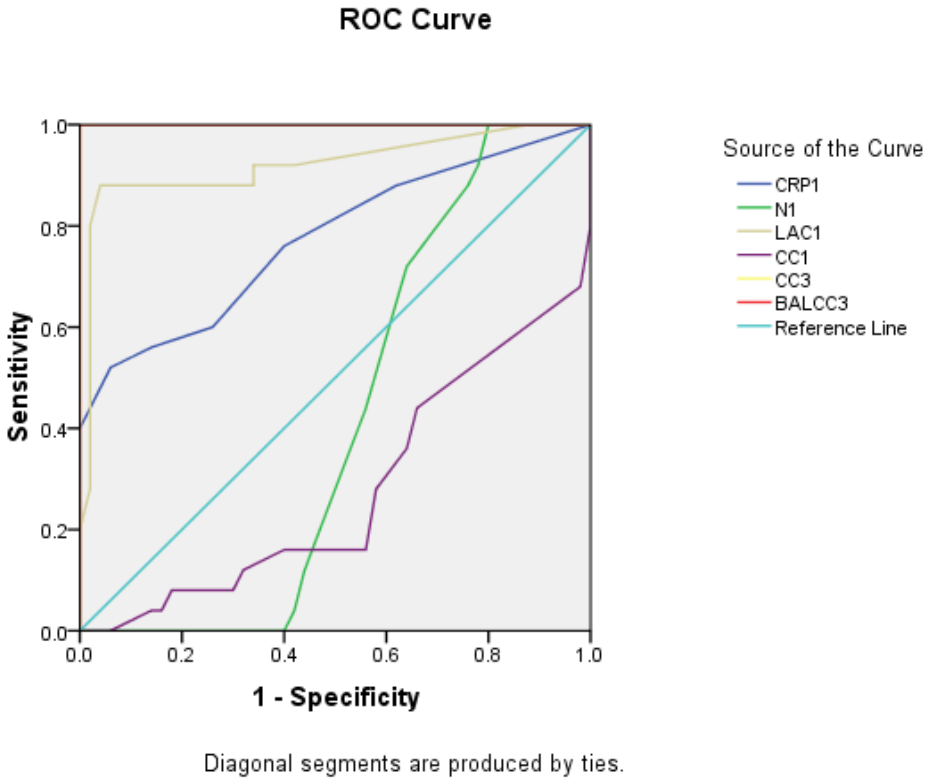
Area under the curve (ROC)	Cut off	p value	Sensitivity	Specificity
1	13.7	0.0001	86%	90%
The markers				
- CRP1	0.77	0.0001		
- N1	0.41	0.234		
- Lac1	0.92	0.0001		
- CC1	0.27	0.002		
- CC3	1	0.0001		
- BALCC3	1	0.0001		

Legend: CC16=Clara cell secretory protein; CRP1=C-reactive protein on day 1; N1=neutrophil-lymphocytic ratio on day 1; Lac1=lactate on day 1; CC1=Clara cell secretory protein on day 1; CC3=Clara cell secretory protein on day 3; BALCC3=bronchoalveolar lavage Clara cell secretory protein on day 3.

Table 9. Frequent of mortality the studied groups regarding mortality

Mortality	Group I (N=75)	Group II (N=75)	Total	p value
Alive	50	70	120	0.001
Dead	25	5	30	

Figure 1. Area under ROC curve for different markers



Legend: CRP1=C-reactive protein on day 1; N1=neutrophil-lymphocytic ratio on day 1; Lac1=lactate on day 1; CC1=Clara cell secretory protein on day 1; CC3=Clara cell secretory protein on day 3; BALCC3=bronchoalveolar lavage Clara cell secretory protein on day 3

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