Value of circulating neutrophil receptors in isolated chest trauma patients: can they predict acute respiratory distress syndrome?

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Background
The dysfunctional immune system is one of the foremost reasons for trauma-related mortality. Severe thoracic injuries are related with serious life-threatening complications such as acute respiratory distress syndrome (ARDS) in spite of ongoing improvements in mechanical ventilation strategies and supportive care; in addition, the surgical intervention can intensify the condition. ARDS is distinguished by the activation of neutrophils and its recruitment into the alveolar space and interstitium of the lung. The exact pathogenesis of this inflammatory complication which follow chest trauma has varied etiologies and the mechanism is not entirely understood. The purpose of this investigation was to assess the neutrophil cells surface receptors expression in severe chest injury and its contribution to ARDS development.

Patients and methods
Blood samples were collected from 50 patients with severe isolated chest injury were examined for the neutrophil cell surface receptors expression profile at a varied interval within the initial 24 h after injury. Patients were followed for the occurrence of any inflammatory complications during this period. For comparison, 25 healthy participants were included as a control group.

Results
Seven patients developed inflammatory complication other than ARDS. Neutrophils showed diminished expression of L-selectin and C5aR and their levels stayed low until 24 h after trauma while both CXCR1 and CXCR2 levels were gradually increased. Furthermore CD11b expression level increased at 3 h and then gradually decreased. Serum level of CXCL8/IL-8 and IL-6 were increased and reach maximum levels after 24 h.

Conclusion
Activation of the circulating neutrophil is transient after isolated chest trauma and leads to a systemic inflammatory reaction to a degree not enough and needs another stimulus to cause ARDS.

Keywords:
chest trauma, neutrophil, surface receptor

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Introduction

Blunt chest trauma has a significant impact on mortality. Around one-quarter of trauma-related deaths is due to severe chest trauma [1]. Patients with chest trauma complications usually deteriorate rapidly and the ability to identify those patients at risk for complications are difficult [2]. Trauma is usually associated mainly with a dysfunctional immune system. One of these immune complications after severe thoracic injury is the acute respiratory distress syndrome (ARDS) [3]. Chest injury induced a systemic inflammatory response with priming and increased circulating neutrophils and release of inflammatory cytokines like interleukin-6 (IL-6) and interleukin-8 (IL-8) [4,5]. Posttrauma surgical interventions usually exacerbate the immune reaction and subsequently increase the incidence of complications, however, the exact mechanism is still obscure [6]. Neutrophils are predominant leukocytes in the human blood. They express a considerable number of cell surface receptors; some of those receptors are able to recognize the inflammatory environment and activate the adaptive immune response [7].

Activation of these receptors leads to complex series of cellular activation, chemotactic, and migration, ended by production and release of various chemokine (CXC) or cytokine [8]. Analysis of the surface neutrophil receptors and the circulating inflammatory markers can determine the degree of systemic inflammation in chest trauma patients [9,10]. That will help to identify risky patients for posttrauma complication and the selection of the best treatment option [10,11]. The aim of the study is to determine the neutrophil cells surface receptors expression in severe chest injury

and its contribution in the occurrence of posttrauma complications especially ARDS.

Patients and methods

Patients

Fifty adult patients with severe isolated chest injury admitted to the Emergency or Cardiothoracic Surgery Department were examined for the neutrophil cell surface receptors expression profile at variable intervals within the initial 24 h after injury. Blood samples were collected from patients at ~3 h after trauma, another sample after 6 h and third sample 24 h after trauma in sodium-heparin coated tubes. Informed consent was taken from all patients or their representatives.

For comparison, 25 healthy persons were included as control group.

Severe chest trauma was defined as any patients with cardiac vascular injury, severe pulmonary contusion, multiple rib fractures, flail chest, tracheobronchial rupture, and moderate or severe hemopneumothorax [12].

Clinical criteria for ARDS 'the American-European consensus definition of ARDS' includes: progressive dyspnea, chest radiography with patchy shadows in the lung, respiratory rate of more than 35 times/min, PO_2 less than 60 mmHg, and the partial pressure of arterial oxygen to fraction of inspired oxygen ratios (PO_2/FiO_2) less than or equal to 200 mmHg [13].

Exclusion criteria included: associated severe injuries in other body areas with thorax that might increase the systemic inflammation, any associated immunological disease, and patients receiving chemotherapy or died within the first day after hospitalization. All patients were followed until discharge for the presence of any inflammatory complications.

The local ethics committee of Assiut University Hospital approved the study.

Methods

Flow cytometry analysis

Flow cytometric analysis of neutrophil receptor expression by using certain monoclonal antibodies 'fluorochrome-labeled' by a fluorescence-activated cell sorter [10].

After lysis, the red cells were incubated with monoclonal antibodies against activation molecules (e-Bioscience , San Diego, CA, USA) for 45 min on the ice including; fluorescein isothiocyanate (FITC)-labeled IgG2a anti-CXCR1, R-phycoerythrin (RPE)-labeled IgG2a anti-CXCR2, FITC-labeled IgG1 anti-L-selectin (CD62L), FITC-labeled IgG2a anti-C5aR, and RPE-labeled IgG1 anti-αM (CD11b).

FITC-labeled IgG1 isotype control, and RPE-labeled IgG2a isotype control were used as a control. Samples were analyzed on flow cytometer (Becton, Dickinson and Co. FACScalibur, Mountain View, California, USA).

The median fluorescence intensity of minimum 10 000 neutrophils in arbitrary units was used for the data presentation.

Plasma inflammatory markers

For CXCL8/IL-8 and IL-6 measurements; plasma samples from patients (at different interval after injury) and controls were stored at −80°C until analysis.

CXCL8/IL-8 and IL-6 were measured by an enzyme-linked immunosorbent assay (ELISA) using Human IL-8/CXCL8 Immunoassay Quantikine ELISA kit (R and D Systems Inc., Canandaigua, NY, USA), and the Thermo Scientific Human IL-6 ELISA Kit, (ThermoFisher Scientific, Waltham, MA, USA) as per the manufacturer's instructions.

Statistical analysis

For data analysis, SPSS (Statistical Package for Social Science SPSS Inc., Chicago, Illinois, USA), 18.0 was used. *P* value less than 0.05 was considered statistically significant. Values were expressed as mean ± SD, number and percentage. For comparison the independent sample *t* test or the Mann–Whitney *U* test was used.

Results

Studied patient characteristics summarized in Table 1.

The study included 50 patients had isolated severe chest injuries their mean age was 50 ± 11 years, 34 of the males and 21 female.

Patients with laboratory result indicating neutrophilic activation from their characteristic surface receptor expression or high plasma inflammatory markers levels were considered at risk for posttraumatic complications. They were advised to delay their surgical intervention to avoid inflammatory complication such as ARDS and to take proper measures to guard against possible infections.

Seven patients experienced one of the inflammatory complications during hospitalization: four patients developed pneumonia and started treatment and three patients developed empyema and underwent tube thoracostomy. While none of the studied chest trauma patients developed ARDS.

Mean hospital stay of the patients was 16 ± 4 days. Four from the total patients their conditions had need of mechanical ventilation for a duration of 5 ± 2.5 days.

Neutrophil cells surface receptor expression

Neutrophils showed diminished expression of both L-selectin and complement receptor 5a (C5aR) compared with control values, their levels stayed low until 24 h after trauma and the differences were statistically significant at 6 and 24 h following trauma ($P < 0.05$) (Table 2).

While both CXC receptors 1 and 2 (CXCR1 and CXCR2) levels were lower than the controls. Then their level starts gradually to increase with time but again still lower than the controls, with no statistical significance for CXCR1 while for CXCR2 the difference was statistically significant.

Moreover, at 3 h after trauma patients showed higher CD11b expression level than the control with no statistical significance ($P > 0.05$) and with time the level start gradually to decrease with a statistical significance difference than the controls ($P < 0.05$).

Plasma level of interleukin-8 and interleukin-6

Both CXCL8/IL-8 and IL-6 plasma levels were increased than their levels in the control group and reach maximum levels after 24 h from trauma with statistically significant differences at all times ($P < 0.05$) (Table 3 and Figs. 1–3).

Table 1 Characteristics of the studied chest trauma patients

	Chest trauma patients (*n*=50)
Age (mean±SE)	50±11
Sex (male/female)	32/18
Mechanism of injury [*n* (%)]	
Motor car accident	29(58)
Fall from height	17(34)
Others	4(8)
Diagnosis [*n* (%)]	
Multiple rib fractures	6 (12)
Flail chest	4 (8)
Pulmonary contusion	5 (10)
Rib fractures+pneumothorax	4 (8)
Rib fractures+hemothorax	12 (24)
Rib fractures+hemopneumothorax	7 (14)
Rib fractures+pulmonary contusion	4 (8)
Flail chest+pneumothorax+pulmonary contusion	4 (8)
Rib fractures+other bone fracture (sternum)	4 (8)

Discussion

High incidence of serious posttrauma complications and mortality is common with severe trauma [8]. The mortality rate increased with the acute lung injury (ALI) and may also with ARDS following severe lung contusion which may be caused by inflammatory cells migration including neutrophils into the lung but the exact mechanism is still unclear [14,15]. Immune suppression and high levels of proinflammatory cytokines in the lung can enhance the risk of ALI [9,15]. Complement cascade activation 'either local or systemic' and the generation of complement component C5a with subsequent activation of the polymorph nuclear cells (neutrophils) which can cause vascular endothelial cells damage and accompanied by ALI [16,17]. The mortality rates remained high after chest trauma despite the new emergent diagnostic procedures and new the treatment modalities in patients with thoracic trauma during the last decades.

Table 2 Receptor expression profile on the neutrophil surface

	Chest trauma patients (*n*=50) [mean (SD)]			Healthy control (*n*=25) [mean (SD)]
	3 h after trauma	6 h after trauma	24 h after trauma	
CXCR1 (MFI)	296.3 (10.7)	290.0 (9.9)	323.8 (9.4)	311.3 (10.9)
CXCR2 (MFI)	45.1 (5.4)*	72.5 (3.2)*	82.5 (3.2)*	180.1 (12.4)
L-selectin (MFI)	294.4 (10.6)	201.3 (10.9)*	190.1 (17.1)*	288.8 (10.1)
C5aR (MFI)	410.2 (8.9)	406.1 (8.9)*	400.0 (9.8)*	507.5 (29.5)
CD11b (MFI)	283.8 (19.1)	152.5 (6.6)*	186.3 (5.5)*	276.3 (19.1)

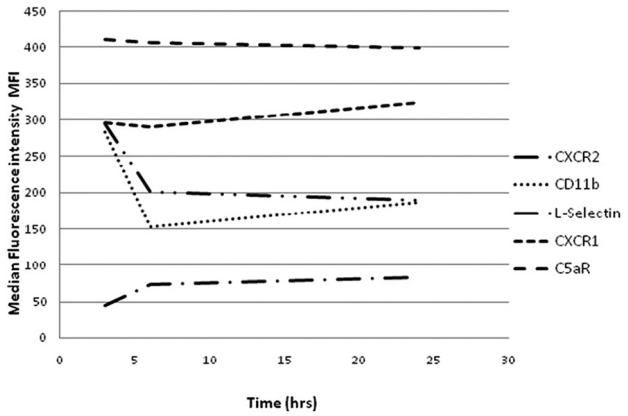
CXC, chemokine; MFI, median fluorescence intensity. **P* value (<0.05) is significant.

Table 3 Levels of the circulating interleukin-8 and interleukin-6 in the studied participants

	Chest trauma patients (*n*=50) [mean (SD)]			Healthy control (*n*=25) [mean (SD)]
	3 h after trauma	6 h after trauma	24 h after trauma	
CXCL8/IL-8 (pg/ml)	40.9 (5.4)*	87.4 (6.1)*	99.5 (9.4)*	1.4 (0.2)
IL-6 (pg/ml)	50.3 (6.2)*	70.0 (6.5)*	93.8 (4.3)*	0.9 (0.2)

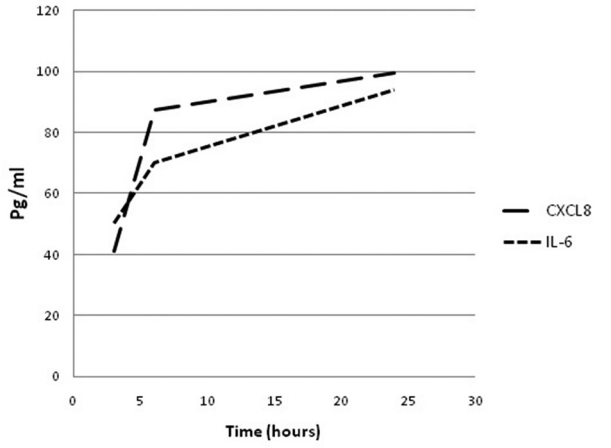
CXC, chemokine; IL, interleukin.**P* value (<0.05) is significant.

Figure 1



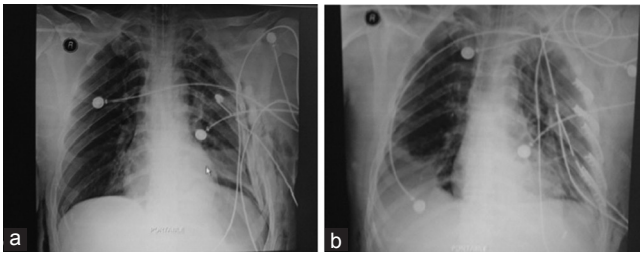
The mean neutrophil surface receptor expression level over time.

Figure 2



CXCL8 and IL-6 mean plasma levels changes over time. CXC, chemokine; IL-6, interleukin-6.

Figure 3



Chest radiography before (a) and after surgical fixation (b) with no sign of inflammatory complication (ARDS) for a patient had multiple rib fractures with pattern of activated surface neutrophil expression the patient was advised to delay the surgical intervention and measures to control infection were taken. ARDS, acute respiratory distress syndrome.

Recently many studies on thoracic trauma revealed that there is a lot of points still unclear regarding the exact role of the inflammatory response in the incidence of posttraumatic complication [18]. The migration capacity of the circulating neutrophils is enhanced within 2–4 h after major trauma with the release of inflammatory mediators [19]. Excess production of mediators such as complement components, cytokines, or CXC usually modulates the function of the circulating neutrophils leading to either immune dysfunction or hyperimmune state according to the type and level of the released mediators [20,21]. Various distinct receptors are displayed on the neutrophil cell surface through which it can respond to numerous inflammatory signals [5]. Study of the expression characterization of the neutrophil cells surface receptors can predict the extent of the inflammatory response. The previous study done by Visser *et al.* [10] denoted that after blunt chest injury the responsiveness of the neutrophil cells due to reduced expression levels of a receptor called L-selectin responsible for neutrophil binding to the endothelium. In addition, the expression levels of both CXCR2 and C5a were also reduced. The stimulated neutrophils can be known by the lowered expression level of L-selectin receptor (also called CD62L), on the other hand, the CD11b (also called αM integrin) receptor expression is upregulated [8,22]. Here we found that severe chest trauma could lead to activation of the circulatory neutrophils which usually accompanied by the release of newly formed neutrophils to the blood this state can be identified the pattern of surface receptor expression.

The level of CXCL8/IL-8 and IL-6 levels were raised in our study population and their level persisted high throughout the first-day posttrauma, this finding is previously reported in cases of major and even minor chest trauma and this could be explained by the enhancement of the inflammatory response [10,14].

The chemokine ligand 8 (CXCL8) is one of the principle neutrophil chemoattractant factors and its levels normally modulate the number of recovered neutrophils in various inflammatory conditions as ARDS. Moreover, the levels of CXCL8 together with the other CXCs found to be positively correlated with disease severity [22,23].

After chest injury, the mobilizations of newly formed young neutrophils usually occur after trauma causing increased number of the circulating neutrophils [24]. The young neutrophils are characterized by reduced responsiveness than the mature cells to an inflammatory stimulus and express αM at much lower levels [25] that explain our results, normally mature neutrophils activation accompanied by upregulation of αM expression while an increased number of the circulating young neutrophils will spuriously decrease its total level of expression after trauma. Another explanation for our result regarding CXCR1, CXCR2 levels where a faster decrease in the level CXCR2 than CXCR1 was found due to the rapid internalization

of CXCR2 [26]. Activated neutrophils after trauma were distinguished by a reduced level of expression of surface receptors like CXCR1 and CXCR2 and C5aR, this state of activation is transient and their levels began to rise slowly throughout the first 24 h [27]. Our result supports the finding that circulating neutrophils are activated after chest trauma and this state of activation not last for a long return to normal after a brief period [10]. Severe chest trauma patients have a risk of subsequent complication like ARDS and multiple organ failure with increased rate of trauma-related mortality, measures like delaying the surgical intervention and measures to guard against infection is taken to all our patients. Despite the enhancement of the systemic immune response found and increased neutrophils activity in our patients none showed ARDS even patients having pulmonary contusion or with severe injury.

Conclusion

Activation of the circulatory neutrophil is transient after isolated chest trauma causing a systemic inflammatory reaction to a degree not enough and needs another stimulus to reinforce the immune response like infection or tissue damage to cause ARDS. The pathophysiology of severe chest trauma inflammatory complication is complex, and both patient and trauma-related factors are implicated. Additional considerations are needed to settle whether the surgical intervention could worsen the outcome and enhanced the frequency of ARDS in patients with severe chest trauma.

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Conflicts of interest

There are no conflicts of interest.

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