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Activation State of the Circulating Neutrophil in Isolated Chest Trauma Patients: Characterization of Surface Receptor Expression

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Abstract

The pathogenesis of inflammatory complication after chest trauma and pulmonary injury is incompletely understood. Injury can trigger a systemic inflammatory response, which leads to pre-activation of neutrophils in blood. The aim of this study was to determine the specific expression profiles of neutrophil receptors in relation to the systemic inflammatory response after chest trauma. Blood samples from fifty patients with isolated thoracic injury were analysed for changes in the neutrophil phenotype within 3, 6 and 24 hours after injury. Study patients was assessed for any inflammatory complications during the first 24 hours. L-Selectin expression remained decreased until 24 hrs while CXCR1, CXCR2 and C5aR levels gradually increased. Expression of FcγRII and expression of the active form were lower in trauma patients, no patients developed ARDS. Thoracic trauma leads to activation of the circulating neutrophils which is transient accompanied by mobilization of young neutrophils into the circulation which leads to systemic inflammatory reactions which need a second stimulus to cause inflammatory complications like ARDS.

Keywords: Chest trauma; Neutrophil; ARDS; Blood; Injury; Pulmonary injury

Introduction

Trauma is a major cause of morbidity and mortality which are mainly due to a dysfunctional in the immune system [1,2]. The acute respiratory distress syndrome (ARDS) is a common complication after severe injury, the incidence of ARDS is found to be low in patients with isolated chest injury [3] and with increased severity of pulmonary contusion the incidence increase [4-6].

Injury can trigger a systemic inflammatory response, an excessive immune reaction occurs, with subsequent priming of neutrophils in the circulation [7] and accompanied by the mobilization of new young neutrophils from bone marrow [8]. Surgical intervention can worsen the inflammatory response, thereby increasing the risk of the posttraumatic inflammatory complications [9], and posttraumatic organ failure [10,11]. The identification of patients at risk for the development of such complications and the mechanisms still unclear [12]. Monitoring for the inflammatory markers and circulating neutrophils surface receptors used to determine the severity of inflammation [13]. These will help in the selection of patients at risk for those complications [14,15] and the choice of best treatment strategy for them [16,17]. Polymorph nuclear leucocytes are one of the important effector cells of the innate immune response. Systemic neutrophil activation is usually occurs within 2 to 4 h after induction of the inflammatory response [18]. Activated neutrophil is characterized by decrease expression of L-selectin (CD62L) and the upregulation of CD 11b (αM integrin) expression [14,19]. Also decreased responsiveness of active FcγRII (CD32); one of the main IgG receptor on neutrophils, more sensitive to priming stimuli than CD11b (αM) especially in patients developed ARDS and correlate better with the post traumatic outcome than other receptor expressions [6,20,21].

The study determines the specific expression profiles of neutrophil receptors as a consequence of severe chest injury and it is relation to the post traumatic inflammatory complications.

Material and Methods

Patients

Blood samples from 50 adult patients suffering from chest injury (with abbreviated injury scale (AIS) and injury severity score [ISS] ≥3]) who were admitted to the emergency department was collected in sodium-heparin coated sterile tubes and cooled immediately. Blood samples were taken at approximately 3,6 and 24 hrs after the injury. Patients with any one of the following was considered as severe chest trauma [22]:

- Severe pulmonary contusion
- Trachea bronchial rupture
- Cardiac vascular injury
- Flail chest resulting from multiple rib fractures
- Hematopneumothorax of moderate volume or above

Exclusion criteria included Patients with injuries with an AIS >2 in other regions than the thorax to reduce the effect of systemic inflammation which caused by tissue damage in other region than the thorax, patients with immunological disease or under corticosteroid or chemotherapy or death within 24 hrs after admission. All patients were followed for the presence of any inflammatory complications. ARDS was assessed according to their clinical criteria; Diagnostic criteria for ARDS were includes progressive dyspnea, respiratory rate of >35 times/min, pO_2 <60 mmHg, and $pO_2/FiO_2 \leq 200$ mmHg; and chest X-ray showing patchy shadows in lung [23].

Blood samples from twenty five apparently healthy subjects are included in the study as a control.

Methods

The following of neutrophil receptor expression were analysed with the use of flowcytometry analysis by a fluorescence-activated cell sorter (FACSCaliber, Becton Dickinson, USA) using a set of fluorochrome-labeled monoclonal antibodies (e-Bioscience, USA) as described by Visser et al. [6] to study the relationship between thoracic trauma and systemic neutrophil activation including:

Fluorescein isothiocyanate (FITC)-labeled IgG1 isotype control, Alexa Fluor 647-labeled IgG1 isotype control, R-phycoerythrin (RPE)-labeled IgG2a isotype control , FITC-labeled IgG1 anti-L-selectin (CD62L), RPE labeled IgG1 anti-αM (CD11b), FITC-labeled IgG2a anti-CXCR1 (CD181a), RPE-labeled IgG2a anti-CXCR2 (CD182b), and FITC-labeled IgG2a anti-C5aR (CD88) , Alexa Fluor 647-labeled IgG1 anti-FcγRIII (CD16), RPE-labeled IgG2b anti-FcγRII (CD32), and FITC-labeled A27, a monoclonal phage antibody, which recognizes active FcγRII (active CD32) [21].

The red cells were lysed using ice-cold isotonic NH4Cl. After that white blood cells were washed and resuspended with phosphate buffered saline (PBS2+) supplemented with sodium citrate (0.38% wt/vol) and pasteurized plasma proteins solution (10% vol/vol) (6). Labeled monoclonal antibodies against activation molecules: L-selectin, αM, CXCR1, CXCR2, C5aR, FcγRII and FcγRIII were added to the resuspended cells and were incubated for 45 min on ice.

After incubation and a final wash, each of the samples were analysed on a FACScalibur flow cytometer (Becton, Dickinson & Co., Mountain View, CA, USA).

For active FcγRII expression, the blood was incubated with FITC-labeled monoclonal phage antibody A27 for 45 min on ice [6]. After staining, red cells were lysed, and expression was measured on FACScalibur.

Data from individual experiments are depicted as the median fluorescence intensity (MFI) of at least 10,000 neutrophils in arbitrary units (AUs) and standard deviation of at least 5.000 events.

Interleukin (IL)-6: the release of IL-6 was measured as an additional marker for inflammation. Plasma samples from patients (at 3, 6 and 24 hrs after injury) and control were obtained and stored at -80°C until further analysis. IL-6 levels were measured by an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's protocol (The Thermo Scientific Human IL-6 ELISA Kit, Thermo Fisher Scientific, USA).

The local ethics committee approved the study, and written informed consent was obtained from all patients or their legal representatives.

Statistical analysis

All data was analysed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA) Statistical significance was defined as a $P<0.05$. Results are presented as means ± standard error. The Mann-Whitney U test was used as appropriate to compare between patients or control values.

Results

Patient demographics

Fifty patients and twenty five control were included in the study. All of the patients had isolated chest injuries with AIS varied from 3 to 5 and the mean ISS 18 ± 2. Thirty four of whom were male and 21 female mean age was 52 ± 10 years.

Receptor expression on the neutrophil surface (Table 1 and Figure 1).

	Patients (n=50)			Control (n=25)
	3 hrs Mean ± SE	6 hrs Mean ± SE	24 hrs Mean ± SE	Mean ± SE
L- Selectin (AU)	296.25 ± 10.7	201.25 ± 10.9*	190 ± 17.1*	288.75 ± 10.1
CD11b/αM (AU)	283.75 ± 19.1	152.5 ± 6.6*	186.25 ± 5.5*	276.25 ± 19.1
CXCR1 (AU)	296.25 ± 10.7	290 ± 9.9	323.75 ± 9.4	311.25 ± 10.9
CXCR2 (AU)	45 ± 5.4*	72.5 ± 3.2*	82.5 ± 3.2*	180 ± 12.4
C5aR (AU)	410 ± 8.9	406 ± 8.95*	400 ± 9.8*	507.5 ± 29.5
FcγRII (AU)	73.75 ± 9.4*	80 ± 8.4*	85 ± 8.4*	190 ± 10.8
Active FcγRII (AU)	452.5 ± 21.4*	438.75 ± 19.4*	372.5 ± 21.4*	1232.5 ± 194.7
FcγRIII (AU)	401.2 ± 15.6*	391.25 ± 10.1*	370 ± 19.6*	1092.5 ± 55.4
IL-6 (pg/ml)	50.25 ± 6.2	70.0 ± 6.5	93.75 ± 4.3	0.9 ± 0.2
*P<0.05				

Table 1: The result of patients and control over time.

The mean hospital stay was 7 ± 3 days. Four patients needed mechanical ventilation for a mean duration of 5 ± 2 days. Only five patients developed an inflammatory complication: Two patients developed sepsis due to empyema and underwent thoracotomy and

Pneumonia was diagnosed in three patients. No patients developed ARDS.

Both L-Selectin and CD11b expression were decreased at 6 hrs postinjury ($p<0.05$) compared to control values. L-Selectin expression remained decreased until 24 hrs ($p<0.05$) (Figure 1).

Reduced surface expression of CXCR1 after 3 hrs and the level of expression was increased than controls after 24 hrs but this difference did not reach statistical significance ($p>0.05$). Circulating neutrophils showed a statistically significant decrease in the expression of chemokine receptors CXCR2 at 3 hrs and both CXCR2 and complement receptor C5aR at 6 hrs postinjury ($p<0.05$), their level gradually increased at 6 hrs until after 24 hrs compared to control values.

Expression of FcγRII and expression of the active form were lower in trauma patients compared to control values after 3 hrs ($p<0.05$), expression of FcγRII slightly increased after 6 hrs but remained lower than the control values until 24 hrs. Expression of FcγRIII was markedly decreased until 24 hr after injury ($p<0.05$).

IL-6 levels

IL-6 levels were increased at 3 hrs and the level was much more incresed after 24 hrs postinjury compared to control values [mean concentration of 50.25 ± 6.2, 93.75 pg/ml ± 4.3 pg/ml versus 0.9 pg/ml ± 0.2 pg/ml ($p>0.05$)] (Figure 2).

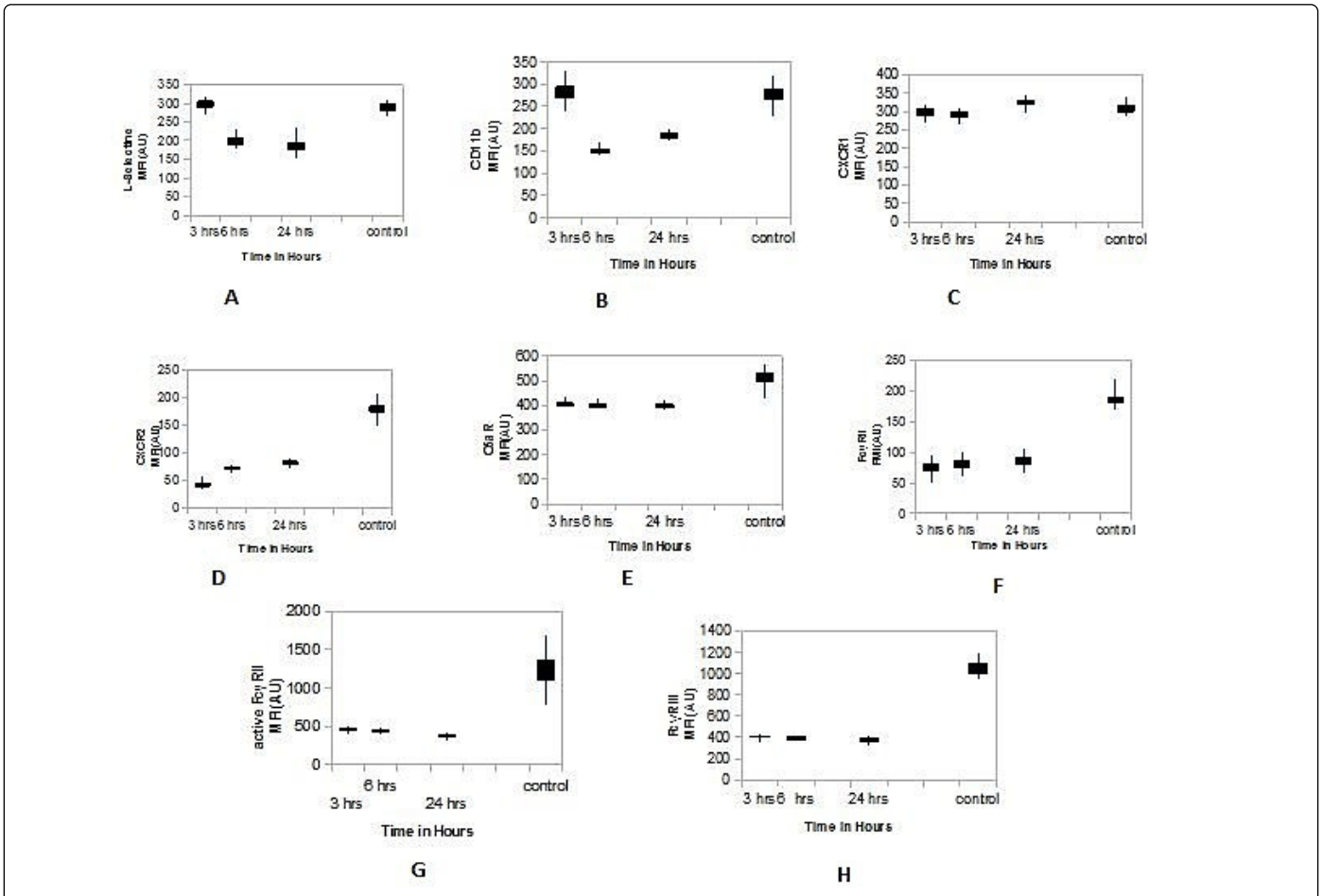

Figure 1: Expression of profile of neutrophil receptors in patients over time and control.

Discussion

Severe trauma to the chest with pulmonary contusion causes approximately a quarter of all trauma related deaths due to a dysfunctional immune system. Neutrophils are essential effector cells, major blunt trauma enhances the migratory capacity of circulating neutrophils within 2 to 4 hrs. Additional trauma caused by surgical intervention can worsen the inflammatory response [14].

Inflammatory mediators are released after trauma even when adult ARDS and MOF (multiple organ failure) do not occur [18]. The modulation of neutrophil function by injury is unpredictable, and can predispose either to hyperimmune states contributes to acute lung injury (ALI), ARDS, multiple organ failure or to immune dysfunction, which permits post traumatic infection, and sepsis. Such outcomes have been related to excess production of the various biological mediators including cytokines, complement (e.g. C5a), CXC chemokine and coagulation factors [4,5].

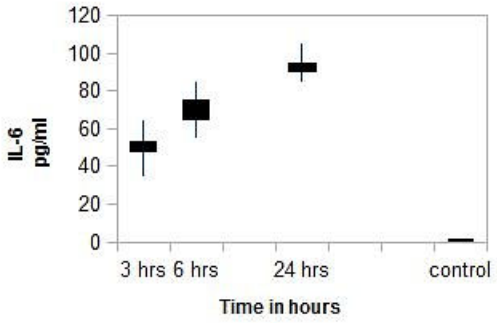

Figure 2: IL-6 level in patients over time and control.

Neutrophils differentially express a specific surface receptors by which the cells can respond and adapt to different signals [11]. The determination of specific expression profiles of neutrophil receptors can aid in quantifying the systemic inflammatory response. This study showed that chest trauma leads to a systemic activation of the neutrophils in the circulation, which is accompanied by the mobilization of new young neutrophils.

Our result come in accordance with Visser et al. [6] who found that after blunt chest injury there was a shedding of L-selectin; a receptor that facilitates tethering of neutrophils to the endothelium and decrease expression of CXCR2 and C5aR and the responsiveness of circulating neutrophils was reduced Maier et al. [3] found that severe lung contusions contributes to an immediate onset of posttraumatic inflammation in severely traumatized patients, resulting in MOF. Neutrophil migration into the lung is a critical, but poorly understood step in the pathogenesis of post-traumatic, ARDS [24]. Elevation of pro-inflammatory cytokines in the lungs with subsequent immune suppression is associated with an enhanced risk for ALI progression [15,24]. None of our patients developed ARDS even those with severe injury or pulmonary contusion that findings were reported an earlier studies [4,6] where the innate immune response provoked was not enough to cause ARDS. This study showed that IL-6 serum level increased in isolated chest trauma patient resulted in an increase in circulating IL-6 the finding that was found in major as well as minor lung contusions due to enhanced inflammatory response, and increased levels of circulating IL-6 and IL-8 in a study performed by Maier et al and increased level of IL-6 in a study performed by Visser et al. [6].

Isolated chest injury is accompanied by the mobilization of young neutrophils where normally FcγRIII is expressed at lower levels than more mature forms [25]. This explain our finding that expression level of FcγRIII reduced after injury until 24 hrs suggesting an influx of young neutrophils with a reduced responsiveness to an inflammatory stimulus. The mobilization of young neutrophils is most likely the cause of increased number of circulating neutrophils after chest injury. This explain the decrease in αM expression although neutrophil activation is characterized by αM upregulation where young neutrophils normally express αM at low levels [26]. CXCR1, CXCR2 and C5aR expression reduced upon neutrophil activation [27] where CXCR2 internalizes faster than CXCR1 [28,29]. Activation of circulating neutrophil was transient after trauma and by 24 hours after injury, the expression of CXCR2 and C5aR was gradually restored [6].

Secondary complications, such as ARDS, MOF and nosocomial infections remain a significant cause of death in hospitalized trauma patients, isolated chest injury rarely leads to ARDS contrasts with conditions like during pneumonia or after aspiration in which the lung is locally affected [25]. An additional stimulus such as concomitant tissue damage, or infection is required to enhance the immune response and increased the risk of developing ARDS.

We therefore suggest that that the systemic immune response resulted from isolated chest trauma is transient and maintained for a short time accompanied by mobilization of new young neutrophils into the circulation but is not sufficient to cause ARDS.

As trauma-induced changes in neutrophil biology have been linked to the development of such common post-traumatic complications, improving patient outcome via the manipulation of neutrophil function or life-span remains an interesting area of investigation in trauma research to reduce immune-mediated tissue damage and the development of post-traumatic complications. However, attempting to dampen down the neutrophil inflammatory response would leave the patient vulnerable to microbial infections. A balance between these two issues should be the focus of future studies.

Conflict of Interest Statement

The authors declare that that they have no conflict of interest.

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