

# CD14+ Monocytic Cytokines: Impact on Outcome in Severely Injured Patients

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## Abstract

**Introduction:** Trauma is one of the leading causes of mortality worldwide. Trauma patients, who presented to the hospital casualty department within 24 h of injury, further admitted to the Surgical Intensive Care Unit were enrolled in this study. **Materials and Methods:** The aim was to study the peripheral blood monocyte activity to produce tumor necrosis factor (TNF- $\alpha$ ), interleukin-1 (IL-1)  $\beta$ , IL-6, and IL-8 in severely injured patients after trauma. **Result:** A total of 28 polytrauma patients were enrolled and observed that the levels of TNF- $\alpha$ , IL-1  $\beta$ , IL-6, and IL-8 were significantly decreased and levels of IL-8 were increased in the fatal patients compared to the healthy controls. **Conclusion:** After trauma, an immediate hyperactivation of circulating monocytes is rapidly followed by a substantial paralysis of cell function. Decreased activity of monocytes can be used to identify potential fatal immunological disruption. Since immunological disruption occurs before clinical symptoms; our study proposes an immunological prognostication score for trauma victims.

**Keywords:** Interleukin-1 beta, interleukin-6, interleukin-8, monocytes, trauma, tumor necrosis factor-alpha

## INTRODUCTION

Trauma is the leading cause of death worldwide.<sup>[1-3]</sup> Mortality in critically injured trauma patients is mainly due to posttraumatic complications. Recent studies indicate that immunological disruptions after traumatic injuries predispose the patients to susceptibility to systemic inflammatory response syndrome, sepsis and multiple organ dysfunction syndrome (MODS), afflicting those who survive the initial resuscitation period.<sup>[3,4]</sup> Trauma usually provokes the production and release of pro-inflammatory cytokines such as tumor necrosis factor (TNF- $\alpha$ ), interleukin-6 (IL-6), IL-1  $\beta$ , and IL-8.<sup>[5]</sup> In most published studies, the immune response of trauma patients has been studied using whole blood or plasma cytokines.<sup>[5,6]</sup> A number of studies have only been limited to biomarkers such as C-reactive protein, procalcitonin, and neopterin as a prognostic or diagnostic marker for posttraumatic complications. None of the biomarkers has been found to be reliable for detection of posttraumatic complications. Most studies are also limited in the follow-up of patients. The present study was designed to ascertain the role of monocytes in the posttraumatic immune dysfunction using flow cytometry. All patients enrolled in the study were followed up to ascertain clinical outcome.

## MATERIALS AND METHODS

A prospective study was conducted at our center, a 152-bedded, level-1 trauma center from July 2014 to July 2015. Trauma patients admitted in hospital casualty within 24 h of injury was enrolled in the study. Patients with isolated head injury, known immunodeficiency states or having received hormone therapy having chronic disease of the liver, kidney, or lung and referred from other hospital were excluded from the study. To categorize the patient's definitions for complication and trauma are given in Table 1. Healthy laboratory and hospital employees of both genders served as the control group. Five milliliter of whole blood were collected from each patient and HCs which further divided equally in heparinized (BD vacutainer lithium heparin 75 USP units, BD Franklin Lakes NJ, USA) and plain vials from all patients on the day of admission and on days 2, 5, and 10 thereafter as follow-ups

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**Table 1: Definition of complication considered in the study**

Complication	Definition
Sepsis	This will be defined as the systemic response to infection. The presence of two or more of the following in a patient suspected or proven to have an infection will be taken as diagnostic of sepsis: 1. Temperature >38°C or <36°C 2. Heart rate >90 beats/min 3. Respiratory rate >20 breaths/min or Pa CO <sub>2</sub> <32 mmHg 4. WBC count >12,000 cells/mm <sup>3</sup> or <4000 cells/mm <sup>3</sup> or >10% immature (band) forms.
Systemic inflammatory response syndrome (SIRS)	The diagnosis of SIRS will be made according to the criteria of the Consensus Conferences of the American College of Chest Physicians and Society of Critical Care Medicine (ACCP/SCCH). Thus, two or more of the following will define SIRS: Body temperature >38°C or <36°C Heart rate >90/min Respiratory rate >20/min Hyperventilation: PaCO <sub>2</sub> <32 mm Hg. WBC count: >12,000/mm <sup>3</sup> / Immature neutrophils granulocytes >10%
Multi-organ dysfunction syndrome (MODS)	Presence of altered organ functions in an acutely ill patient, such that homeostasis can-not be maintained without intervention is known as MODS. <sup>[5]</sup> MODS will be defined according to the method described by Marshall <i>et al.</i> Manifest MODS will be considered at a marshall score of >12 points on two consecutive days or at least 3 days during the observation period. MODS will be assessed on admission, and daily till the discharge/death of patient
Polytrauma	Two or more severe injuries in at least two areas of the body

to ascertain the patient's clinical outcome and correlate it with monocytic activity. The whole blood was challenged with 1 µg/mL lipopolysaccharide (LPS) (*Escherichia coli*, Serotype 055:B5, Sigma-Aldrich, St Louis, MO, USA) immediately after collection and incubated at 37°C in 5% carbon dioxide with 1 µg/mL monensin (BD, San Jose, CA, USA) to inhibit protein secretion for 4 h, basal (unstimulated) tube without LPS was also treated similarly. After stimulation surface staining with CD45 and CD14 tagged with PerCP Cy5.5 and phycoerythrin (PE)-Cyanine7 (BD, San Jose, CA, USA) respectively was done which is followed by intracellular staining by specific antibodies for TNF-α, IL-6, IL8, and IL-1 β tagged with PE, allophycocyanin, and fluorescein isothiocyanate, respectively (BD, San Diego, CA, USA). After washing, the pellet was resuspended in 1% paraformaldehyde (Fisher-Scientific, Mumbai, India). The cells were then analyzed by flow cytometry.

All the patients enrolled in the study were clinically followed up till their final hospital outcome (discharge/death). Details of surgical procedure performed, antimicrobial treatment and clinical as well as autopsy reports of fatal cases to find the cause of death were similarly noted. Results were recorded as mean ± standard error of the mean. Data were analyzed by the Mann-Whitney Test for multiple comparisons.

A total of 28 polytrauma patients were included in the study. The average ISS score was 6–45. Of the 28 trauma patients, 4 (14%) had isolated abdominal trauma, 3 (10.7%) had isolated chest trauma, 3 (10.7%) had maxillofacial injury, 6 (21.4%) had chest and abdominal trauma, and 12 (42%) had polytrauma. The demographic details of patients enrolled in the study are given in Table 2.

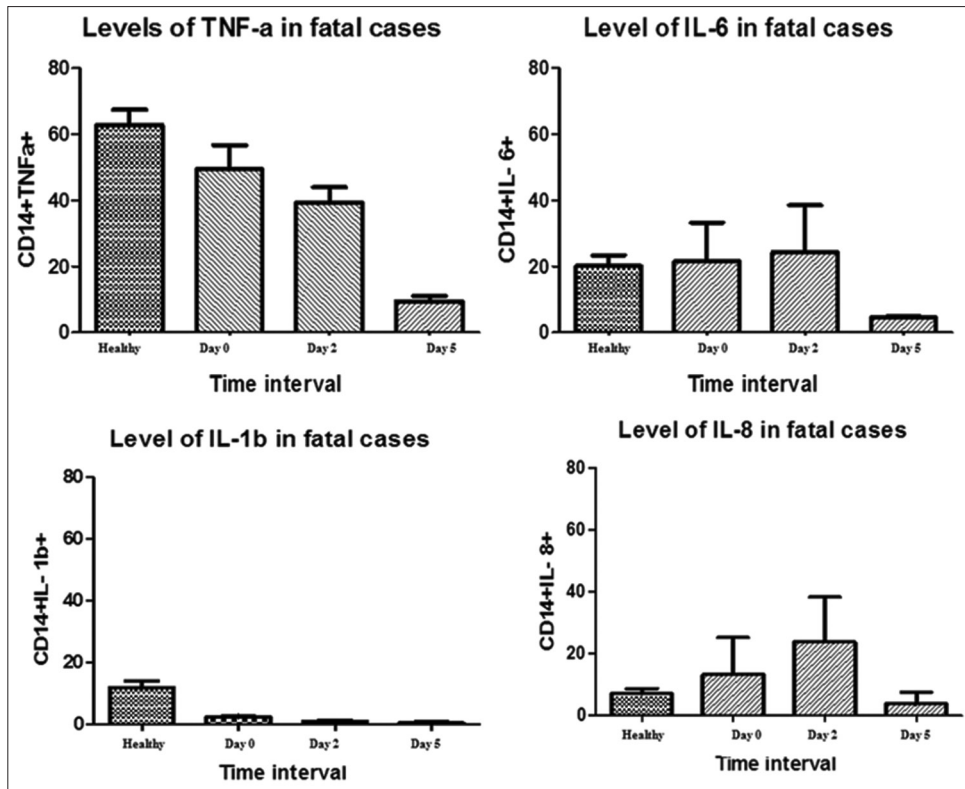
Among the 28 patients, posttraumatic complications such as MODS, VAP, septicemia, and ARDS were seen in 4 (14.2%)

patients. Of these, two cases were fatal and the other two recovered and were discharged following variable lengths of stay in the hospital. All four patients had ISS between 34–38 and were also ventilated. A total of 9 (32%) patients, of the total, enrolled patients ( $n = 28$ ) had a positive culture from different samples. Bronchoalveolar lavage fluid and blood were the most common clinician samples and *Acinetobacter baumannii* and *Pseudomonas aeruginosa* were observed to be the most common organisms in the culture-positive samples as shown in Figure 2 [details given in Table 3].

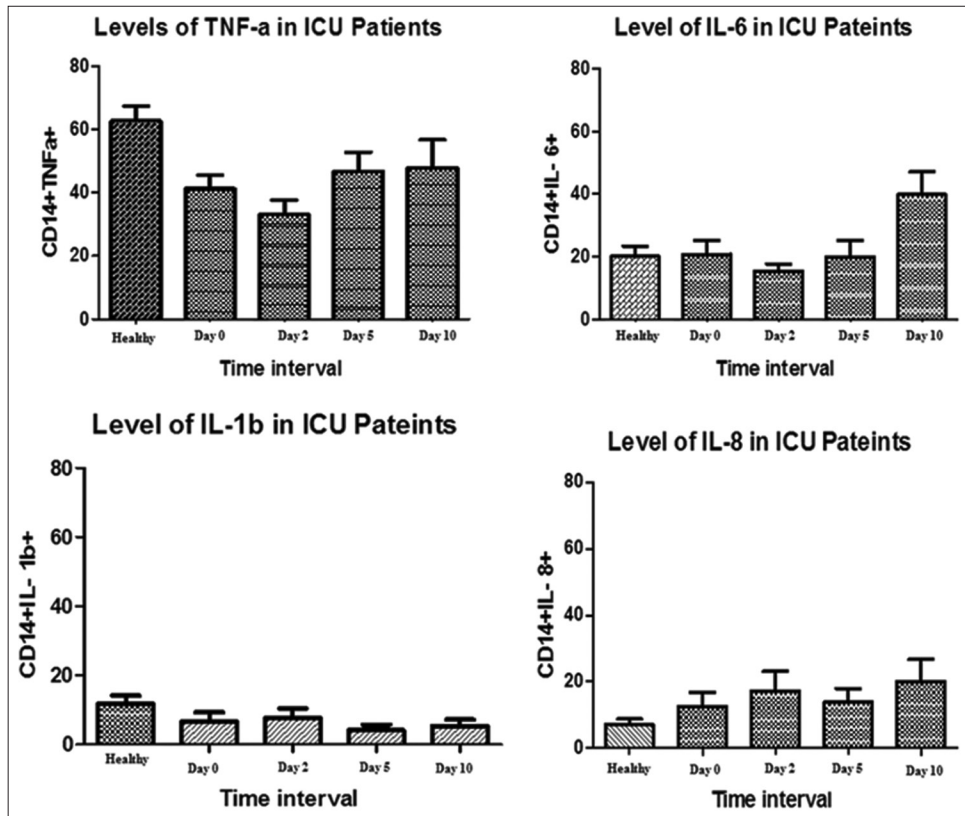
The study revealed the absence of monocytic cytokine producing activity in patients and HCs under unstimulated conditions. However, after 4 h of stimulation with LPS, the cytokines levels were observed to be elevated in HCs (CD14+ TNF-α+62.74 + 4.6, CD14+IL-6 + 21.03 + 3.4, CD14+ IL-1 β+12.20 + 2.6, and CD14+ IL-8 + 7.15 + 1.7) as shown in Table 4.

In Intensive Care Unit (ICU) patients, the levels of TNF-α were found to be lower than those in HCs, immediately after the injury (day 0) which subsequently decreased till day 5. The levels of TNF-α CD14+ TNF-α+47.9 + 8.8) increased to levels comparable to those in HCs by day 10 postinjury, as shown in Figure 1. In the four fatal cases, the levels of TNF-α were found to be lower than those in HCs in the initial period of trauma (CD14+ TNF-α+49.53 + 7.1). These levels significantly decreased (CD14+ TNF-α+9.5 + 1.5) as the patient's clinical condition started to deteriorate.

Similarly, the levels of IL-6 in ICU patients were found to be lower than those in HCs immediately after the injury (day 0) which subsequently decreased till day 5 and increased to levels comparable to those in HCs, by day 10 (CD14+ IL-6 + 39.86 + 7.26) postinjury, as shown in Figure 1.



**Figure 1:** Levels of intracellular cytokine produced by monocytes within the first 24 h of trauma till the outcome, compared with healthy control. Statistical data are given as graphs. All compared with healthy control.  $P < 0.05$  considered as statistically significant



**Figure 2:** Levels of intracellular cytokine produced by monocytes within the first 24 h of trauma and on day 2, 5, and 10, compared with healthy control. Statistical data are given as graphs. All compared with healthy control.  $P < 0.05$  considered as statistically significant

The levels of IL-1  $\beta$  were found to be lower than those in HCs, immediately after the injury which was found to decrease till day 5. However, the levels of IL-1  $\beta$  were found to be nearly similar to those in HCs in all the further follow-ups. The statistically significant lowest mean values were observed on day 5 postinjury.

In contrast, the levels of monocytic IL-8 were markedly high on day 0 (CD14+ IL-8 + 12.64 + 4.09) ( $P=0.2367$ ), which increased by day 2 (CD14+ IL-8 + 17.12 + 6.06) ( $P=0.1153$ ) and slightly decreased by day 5 (CD14+ IL-8 + 13.83 + 4.11) ( $P=0.1094$ ), and again significantly increased by day 10 (CD14+ IL-8 + 20.15 + 6.5) ( $P=0.132$ ). The IL-8 production was higher than that in HCs on day 0, but a dip was observed by day 5.

### DISCUSSION

The present study focused on the monocytic immunomonitoring of the polytrauma patients admitted to the ICUs for early

diagnosis of posttrauma complications for ICU. Till now, most of the studies have only explored these inflammatory responses in the animals models,<sup>[7]</sup> thus, the same questions pertaining to humans remain unanswered. Moreover, the proinflammatory roles of TNF- $\alpha$ , IL-6, IL-1  $\beta$ , and IL-8 are well known, but their anti-inflammatory roles are now being understood. In this study, the levels of monocytic TNF- $\alpha$  were observed to be significantly decreased immediately posttrauma, which however were upregulated by day 10 in all the patients who were discharged. Those with a poor outcome had a significant and gradual decrease in the monocytic TNF- $\alpha$  levels. Studies have contrastingly shown IL-6 to be directly or inversely correlated with poor outcomes.<sup>[8]</sup> We found that the monocytic IL-6 production behaved in a manner similar to the TNF- $\alpha$  production in the ICU patients.

IL-1  $\beta$  and IL-8 is an inflammatory cytokine and barely detectable in the serum. High IL-1ra/IL-1  $\beta$  ratio has been associated with better outcomes.<sup>[8]</sup> In our studies, the levels of intracellular IL-1  $\beta$  and IL-8 was observed to be significantly increased by day 10 and positively correlated with a favorable final outcome and decreasing levels with a poor outcome. Therefore, these cytokines can also be used as a prognostic marker.

There are very few studies on monocytic activity in trauma patients. Kirchhoff *et al.* studied the monocytic activity in early phase trauma and reported that the levels of TNF- $\alpha$ , IL-6, IL-1  $\beta$ , and IL-8 decreased as compared to HCs after 6 h of trauma but tend to normalize by 3 day (72 h).<sup>[9]</sup> They did not study the cytokine profile post 72 h. In another study,

**Table 2: Demographic data of study population**

	ICU patients	Fatal cases	VAP patients	MODS patients
Number of patients	28	4	2	2
Age	31 $\pm$ 12	33 $\pm$ 4	29 $\pm$ 0.7	40 $\pm$ 8
ISS	21.3 $\pm$ 10	23 $\pm$ 12	30 $\pm$ 11	29 $\pm$ 11
ICU stay	5 $\pm$ 4	12 $\pm$ 11	9 $\pm$ 6	10 $\pm$ 7
Ventilated days	5 $\pm$ 6	12 $\pm$ 11	6 $\pm$ 4	10 $\pm$ 7
Hospital stays	13 $\pm$ 9	12 $\pm$ 11	9 $\pm$ 6	10 $\pm$ 7

**Table 3: Microbiology analysis of study population**

ISS	Sample	Organism	Ventilated days	Complications	LOS	Outcome
13	BAL	<i>P. aeruginosa</i> <i>P.stuartii</i>	14	NA	24	Discharged
11	Tracheal	<i>P.aeruginosa</i>	16	NA	20	Discharged
38	BAL	<i>P. aeruginosa</i>	10	ARDS	10	Fatal
45	BAL	<i>A.baumannii</i> <i>K.pneumoniae</i>	5	NA	20	Discharged
35	Blood	<i>A.baumannii</i>	2	MODS, VAP, Septicemia, CLABSI	20	Discharged
36	Pus	<i>A. baumannii</i>	12	NA	10	Discharged
38	BAL	<i>A.baumannii</i>	5	NA	5	Discharged
45	Pus	<i>Paeruginosa</i>	12	A	5	Discharged
34	BAL & Blood	<i>A.baumannii</i>	20	Knee amputation, MODS, VAP, Septicemia	20	Fatal

**Table 4: Statistical results of Monocytic cytokines level in different group of fresh trauma Patients**

Time	TNF-a			IL-6			IL-1b			IL-8		
	Control	ICU patients	Fatal cases	Control	ICU patients	Fatal cases	Control	ICU patients	Fatal cases	Control	ICU patients	Fatal cases
HCs	62.74+4.6			21.03+3.4			12.20+2.6			7.15+1.7		
Day 0	41.29+4.2**	49.5+7.1		20.86+4.27	21.53+11.8		6.57+2.7	2.75+0.7		12.64+4.09	13.25+11.9	
Day 2	33.18+4.5***	39.37+4.5		15.30+2.41	24.37+14.13		7.71+2.71	1.03+0.4		17.12+6.06	23.25+14.64^^	
Day 5	46.8+5.9*	9.5+1.5~		19.95+5.27	4.55+0.4		4.07+1.68 <sup>s</sup>	0.46+0.4		13.83+4.11	3.75+3.75	
Day 10	47.9+8.8			39.86+7.26 <sup>#</sup>			5.34+1.85			20.15+6.5^		

Spolarics *et al.* also studied late stages of trauma, but there were no fatal cases in either of the studies.<sup>[10]</sup> Our study sheds light on the correlation of the monocytic cytokine levels and adverse outcomes.

However, the study could have been strengthened if lymphocytic activity was also studied.

Our data might contribute to a better understanding of the role of immunomodulations over time after traumatic injuries since our results were derived from wide intervals of measurements up to 240 h posttrauma (day 10).

It can be concluded that trauma provokes an immediate hyperactivation of circulating monocytes in the early phase followed by a substantial paralysis of cell function leading to a compromised cytokine-producing capacity till the 5 days' posttrauma and revived by day 10, which results a favorable outcome. The downregulation of monocytic TNF- $\alpha$ , IL-6, and IL-1  $\beta$  as well as significant upregulation of IL-8 leads to poor outcomes. There seems to be an antagonistic action of IL-8 and IL-1  $\beta$  and their correlation with the patient's outcomes. Nevertheless, decreased activity of monocytes can be used to identify potential fatal immunological disruption in patients after trauma. Since immunological disruption occurs before clinical symptoms; our study proposes an immunological prognostication score for trauma victims. More studies with a larger number of polytrauma patients may be required our knowledge about critically injured patient's immunological substantial.

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Nil.

#### Conflicts of interest

There are no conflicts of interest.

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