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Research Article

Serum level of IL-6, IL-10 and PCT as a prognostic marker in post traumatic sepsis patients.

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Abstract

Background: Trauma hemorrhagic shock (THS) leads to multiple organ failure (MOF) with sepsis and without sepsis. The authors studied the pattern and prognostic value of serum cytokines, chemokine, CRP, HMGB1 and PCT levels in patients fallowing THS. **Materials and methods:** Serum levels of cytokines and other markers were determined on admission (Day 0) and 3, 7 and 14 days in patients and 50 healthy controls. Correlation between serum cytokine levels and SOFA score were calculated in order to understand the importance of these cytokines to multiple organ failure (MOF).**Results:** Serum levels of TNF-α was found to be low and IL-10 levels were found to be high on day 0 in those THS patients who were died as a result of sepsis in the ICU. Serum levels of CRP and procalcitonin were found to be elevated in those THS patients who developed sepsis. But procalcitonin level was found to be elevated in death patients due to sepsis and discriminate the sepsis patients with severe sepsis. Our data show that serum cytokine disturbance patterns have prognostic significance in sepsis patients. **Conclusion:** The significantly elevated serum level of IL-6, IL-10 and PCT, is associated with enhanced risk of fatal outcome for THS patients.

Keywords: Injury severity score, Sequential organ failure assessment assay, Shock Index, Trauma hemorrhagic shock (THS).

Introduction

Trauma hemorrhagic shock (THS) is a pathologic state in which intravascular volume and oxygen delivery are impaired. As long as this bleeding is not controlled, the physician must maintain oxygen delivery to limit tissue hypoxia, inflammation and organ dysfunction. This procedure involves fluid resuscitation, use of vasopressors and blood transfusion to prevent or correct traumatic coagulopathy [1]. Trauma complicated with multiple organ dysfunction syndromes (MODS) is a leading cause of death and disability in the intensive care units with mortality rate exceeding 50% [2] . Those who survive the initial hours have additional life-threatening risk of developing, SIRS, sepsis with and without MODS. Post-traumatic sepsis, arises as a result of inappropriate immune inflammatory responses, is considered as one of the commonly observed problems associated in THS patients. The early diagnosis of sepsis and evaluation of its severity is highly variable due to non-specific nature of the signs and symptoms [3]. However, early diagnosis and stratification of the severity of sepsis is critical for starting early goal directed therapy. The immunological response to THS involves not only inflammatory cytokines but also anti-inflammatory cytokines, humoral and cellular reactions and circulatory abnormalities [4, 5]. Despite the use of modern antibiotics and resuscitation therapies, sepsis is one of the major causes of death in critically ill patients [6]. The rise of antibiotic resistance is also a

major challenge that calls for novel bio-markers to guide and limit drug usage. So, here it is important to understand the profiling of serum cytokines at different time points in THS patients and correlates with disease severity.

We hypothesized that comprehensive profiling of serum levels of multiple cytokines would provide greater insight into their utility for staging patients with sepsis, compared with previous studies focusing on a single biomarker. In this study, we quantitatively analyzed 13 markers including serum cytokines, chemokines, procalcitonin (PCT), C-reactive proteins (CRP) and High mobility group box 1(HMGB-1) protein in serum samples from 96 hemorrhagic shock patients on different days (0, 3, 7 and 14) after haemorrhage and outcomes of patients were observed in the form of death with and without sepsis. Secondly, we observed when do the trauma hemorrhagic shock patients develop the sign of sepsis and what are the changes in the serum levels of cytokines in these patients in comparison with those who did not develop sepsis. The final outcome of patients in the form of death and survival was observed till 30 days of observational periods.

1.1 Patients and methods:

1.1.1 Patient's characteristics:

Patients of either sex with age 16-60 years with systolic blood pressure (SBP ≤90 mmHg), presenting within the 8 hr of trauma were recruited after taking the consent from the patients relatives. Those patients who had neurogenic shock, traumatic brain injury (TBI), septic shock and already resuscitated with fluids (colloids or crystalloids), anti-inflammatory drugs or corticosteroids and blood or blood components before reporting to the Emergency Department (ED) were excluded from this study. Blood samples of those THS patients who have SBP ≤90 mmHg were collected at different time points on days 0, 3, 7 and 14. Severity was assessed by Injury severity score (ISS) and Shock Index (S.I) in the Emergency Department and the development of organ dysfunction was assessed by the Sequential Organ Failure Assessment score (SOFA; range, 0–24). Mortality was defined as those patients who died up to 30 days after the onset of hemorrhagic shock. Patients were classified as a survivor if they were discharged alive from the ICU.

Further, we have categorised the patients in two broad categories those who developed sepsis complication and other those who did not during the 30 days of observational period. The inclusion, exclusion criteria along with distribution of patients were shown in consort diagram (Fig. 1S, Supplementary result).

1.1.1.2 Sample Collection: - Collection of First Blood Sample: All patients were managed according to the protocol of American College of Surgeons Advanced Trauma Life Support (ATLS).Patients were stabilized (SBP=105 mmHg) with crystalloids but without blood or blood components for the first sample, which was collected on the Day 0 in the ED.

1.1.1.3 Consecutive Blood Samples were collected on days 3, 7 and 14 irrespective of blood transfusion. Sequential blood sampling was terminated in patient who refused for further sampling or if patients transferred to another hospital.

Blood samples were also collected from 50 healthy, age matched, uninjured individuals who formed the control group for analysis of serum cytokines. Blood samples were collected; serum was separated and stored at -20^{0}C for cytokine estimation.

1.1.1.4 Definitions, Scoring of Severity, and Outcome: Abbreviated injury scale (AIS) scores of traumatic injuries induced while living and diagnosed by autopsy were determined using the 1985 protocol of the American Association for Automotive Medicine. AIS scores range from 1 to 6; total AIS scores, which represent the sum of scores for each body region (head, neck, face, chest, abdomen, extremities and external), and the injury severity score (ISS), [7] which is defined as the sum of the square of the single highest AIS score in each of the three most severely injured body regions, were determined for each case. Shock Index (S.I) is the ratio of Heart rate and Systolic blood pressure (SBP) were calculated for every patients in the Emergency department to understand the physiological injury score of the patients.

The Sequential Organ Failure Assessment score, or just SOFA score, is used to track a patient's status during the stay in an intensive care unit (ICU). The SOFA score is a scoring system to determine the extent of a person's organ function or rate of failure [8, 9]. The score is based on six different scores, one each for the respiratory, cardiovascular, hepatic, coagulation,

renal and neurological systems. The SOFA score was calculated on 7th (SOFA-7) and 14(SOFA-14) days in the ICU using previously described criteria [8].

Systemic Inflammatory Response Syndrome (SIRS): The systemic inflammatory response to a wide variety of severe clinical insults, manifested by two or more of the following conditions:

1) Temperature > 38°C or < 36°C
2) Heart rate > 90 beats/min
3) Respiratory rate > 20 breaths/min or $PaCO_2$ < 32 mm Hg
4) WBC count > 12,000/mm^3 , < 4000/mm^3 , or > 10% immature (band) forms.

Sepsis

The systemic inflammatory response to infection. In association with infection, manifestations of sepsis are the same as those previously defined for SIRS. It should be determined whether they are a direct systemic response to the presence of an infectious process and represent an acute alteration from baseline in the absence of other known causes for such abnormalities. The clinical manifestations would include two or more of the following conditions as a result of a documented infection.

Severe Sepsis

Sepsis (SIRS) associated with organ dysfunction, hypoperfusion, or hypotension. Hypoperfusion and perfusion abnormalities may include, but are not limited to, lactic acidosis, oliguria, or an acute alteration in mental status.

Septic Shock. A subset of severe sepsis (SIRS) and defined as sepsis (SIRS) induced hypotension despite adequate fluid resuscitation along with the presence of perfusion abnormalities that may include, but are not limited to, lactic acidosis, oliguria, or an acute alteration in mental status. Patients receiving inotropic or vasopressor agents may no longer be hypertensive by the time they manifest hypoperfusion abnormalities or organ dysfunction, yet they would still be considered to have septic (SIRS) shock.

Multiple Organ Dysfunction Syndrome (MODS).

Presences of altered organ function in an acutely ill patient such that homeostasis cannot be maintained without intervention.

The presence of infection was defined by previously described strict diagnostic criteria of the American College of Chest Physician/Society of Critical Care Medicine [10].

1.1.1.5 Quantification of serum analytes:

Nine cytokines IFN-γ, IL-1β, TNF-α, TGF-β, IL-2, IL-4, IL-6, IL-10, IL-12 and one chemokines [MCP-1],were assessed using cytometric bead array (CBA) kit (Ten-Plex Antibody Bead Kit, BIOSOURCE, USA) and serum levels of high sensitive CRP, PCT and HMGB-1 were also measured by ELISA.

1.1.1.6 Statistical Analysis:

Statistical analyses were performed using SPSS for Windows 10.0 (SPSS Inc., Chicago, IL, USA) and Graph Pad Prism version 3.0 for Windows (Graph Pad Software, San Diego, CA, USA). Numeric variables are expressed as median (interquartile range) and were assessed using Mann-Whitney *U*-test and Kruskal-Wallis test. Dichotomous variables were analyzed using $\chi 2$ and Fisher's exact test (with Yates correction as indicated). Spearman analysis was employed to detect correlations among continuous variables.

2. Results:

114 patients were recruited for this study. 18 (15.7%) patients were excluded as, 5(27.7%) patients had cardiovascular diseases and diabetes mellitus, 7 (38.8%) had traumatic brain injury (TBI) and 6 (33.3%) patients had already resuscitated with fluids before reporting to ED. 96 (84.2%) patients were included in the final analysis. Out of 96, 25 (26%) patients developed sepsis in our study. Out of 25 patients with sepsis, 8 (32%) developed severe sepsis and 6 (24%) developed septic shock. Clinical profiles of these patients were shown Table 1. Age and S.I in patients with sepsis and in those who did not develop sepsis were comparable (Table 1). Mortality was high in patients with sepsis (64%) when compared to those who did not have sepsis (29.5%) ($p<0.05$).

Table-1 Clinical characteristics of trauma patients those who develop sepsis complication and other didn't (n=96)

	Sepsis	Non-sepsis	P value
Patients	n=25	n=71	
Sex (M/F	18/7	62/9	
Age (yrs)	37.5±16.1	33.4±13.9	0.21
ISS	21.2±6.8	18±8.8	0.05
S.I	1.2±0.44	1.16±0.53	0.5
SOFA-7	5.28±1.96	3.78±2.34	0.006
SOFA-14	7.86±2.18	4.77±2.31	0.0001
Mortality n (%)	16(64)	21(29.5)	0.001

2.1 Comparison of serum cytokines levels at different time points in patients who developed sepsis with other who didn't.

All the serum cytokines, chemokine and other biomarkers which depicted difference between both groups were elucidate in Table 2 and rest of cytokines were shown in supplementary Table S1. The serum level of TNF-α was found to be significantly low ($p= 0.015$) on day 0 in patients who developed sepsis. However, serum levels of TNF-α were found to be elevated ($p<0.05$) on day 7 and 14 in patients who developed sepsis, but its level were reduced in patients who didn't develop sepsis (Table 2). Out of 25 patients with sepsis, 13 (60%) had persistently elevated levels of IL-6 and IL-10 up to day 3 (Table 2). The overall serum levels of MCP-1 and TGF-β were elevated in the sepsis group. The serum level of CRP, PCT and HMGB1 were significantly elevated ($p<0.05$) in sepsis groups as compared to non-sepsis group at days 3, 7 and 14 (Table 2).

2.2 Serum level of cytokines in sepsis and severe sepsis patients:

Serum levels of TNF-α, IL-6, IL-10, MCP-1, TGF-β, PCT and CRP showed significant changes in sepsis and severe sepsis group on day 14 (Fig. 1). Among these, only serum levels of TNF-α, IL-6 and PCT were observed as good prognostic markers of sepsis. These However, these levels were observed significantly elevated on day 7 and it showed positive correlation three markers showed correlation with severity of disease and outcome of the patients. Out of 14 patients with severe sepsis, four (28.5%) patients who had TNF-α ≥400pg/ml died. Serum levels of IL-6 were higher in severe sepsis as compared to sepsis (Fig.1b) and showed positive correlation with SOFA score. Serum level of PCT were in severe sepsis (52.7±26.4 ng/ml) significantly ($p<0.05$) high as compared with sepsis (13.7±9.5 ng/ml) (Fig.1f).It showed positive correlation with SOFA score in the ICU (Fig.2).

2.3 Cytokines, chemokines and other biomarkers in patients with sepsis among survivors and non-survivors.

Sepsis group among non-survivors had low levels of TNF-α on day 0 ($p<0.05$) in comparison to survivors. However, these patients had elevated levels on days 3, 7 and 14 (Table 3).

In comparison to survivors, non-survivors patients had elevated level of IL-10 up to 72 hrs.

It showed positive correlation with SOFA score in patients with sepsis (Fig. 2). Out of 16 non-survivors, 10 (62.6%) had approximately 10-12 fold high serum level of IL-10 on day 0. Serum level of MCP-1 was 19.4(16.2-57.6) pg/ml significantly ($p<0.05$) low in healthy controls in comparison to survivors and non-survivors on days 3, 7 and 14.
with SOFA score (Fig.2).We have observed persistently elevated level of PCT in non-survivors

when compared to survivors, except day 0 (Table 3) and it showed positive correlation with SOFA score on days 7 and 14 (Fig. 2).

2.4 Cytokine concentrations and evaluation of organ failure:

There was a positive correlation between serum levels of cytokines PCT, IL-6, IL-10 and MCP-1 (r = 0.71, 0.80, 0.79 and 0.79 respectively; $P < 0.0001$) on day 7 and with PCT and IL-6 (r =0.78, and 0.62 respectively, P<0.001) on day 14 (Fig. 2).

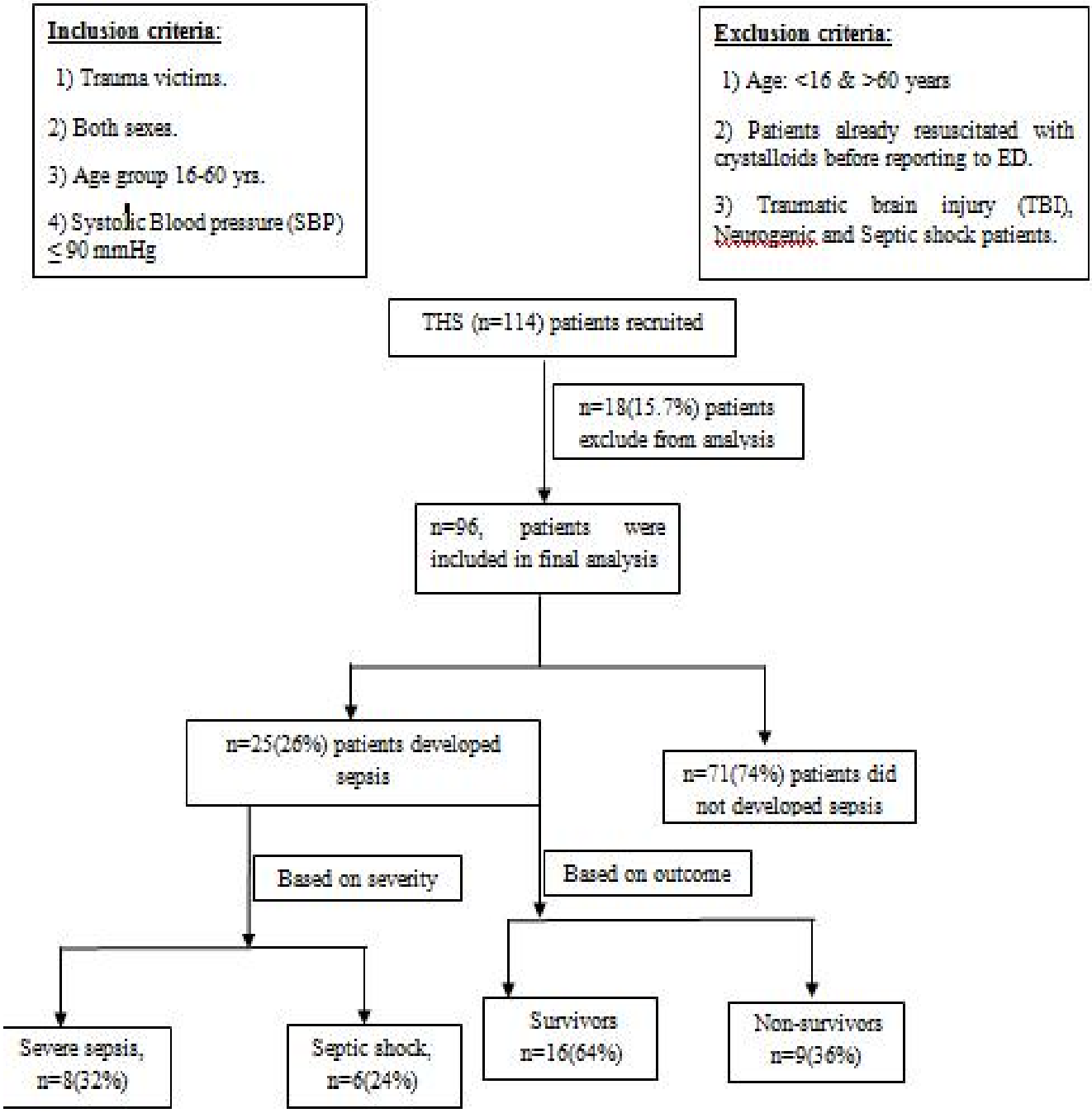

Table 2, Comparison of serum cytokines levels between hemorrhagic shock patients who developed sepsis with other didn't.

Cytokine (pg/ml)		Day 0 Median (Range) (n1:25,n2:71)	Day 3 Median (Range) (n1:25,n2:71)	Day 7 Median (Range) (n1:25,n2:71)	Day 14 Median (Range) (n1:25,n2:71)
TNF-α	Sepsis	335.5 (67.6-1415.8)	320 (16-847)	275.8 (42.3-780.5)	302.6 (16.1-528.8)
	Non-sepsis	525.8 (104.7-986.4)	227.6 (16.3-772.9)	140.7 (39.4-671)	84.7 (22.6-181.4)
P value		0.015	0.06	0.007	0.001
IL-6	Sepsis	287.81 (50.2-539.3)	315 (74.4-1054.3)	139.3 (89.6-954.8)	367.2 (98.7-958.3)
	Non-sepsis	88 (33.2-582)	90 (70.2-1035)	138.2 (50.2-1024.7)	139.3 (101-554.5)
P value		0.02	0.01	0.42	0.04
IL-10	Sepsis	351.5 (25.6-794.4)	194.4 (35.3-1139.4)	154 (95.96-535.4)	158.7 (18.2-606)
	Non-sepsis	122.9 (17.6-1198.8)	137.4 (8.8-575.7)	145 (18.2-546.8)	146 (25.7-345.7)
P value		0.03	0.07	0.24	0.09
MCP-1	Sepsis	23.5 (10.8-72)	52.8 (19-102.6)	177.3 (78.6-256.4)	206 (136.5-293.5)
	Non-sepsis	18.7 (10.8-57)	46.2 (17.6-91.6)	102.5 (62.8-204.6)	110.2 (57.9-152.8)
P value		0.48	0.43	0.07	0.002
TGF-β	Sepsis	13.6 (4.9-26.8)	87.6 (43.8-138.7)	92.8 (37.8-127.5)	123.5 (78.2-203.8)
	Non-sepsis	10.4 (2.8-21.5)	21.5 (7.8-42.3)	28.9 (9.6-51.2)	57.7 (26.5-76.4)
P value		0.39	0.02	0.018	0.04
CRP(mg/L)	Sepsis	67.7 (21-97.5)	78.9 (28.7-142.6)	191.7 (134.8-234)	201.8 (178.6-265.8)
	Non-sepsis	56.5 (15.1-104.8)	63.9 (25.5-101.6)	74.8 (22.8-104.8)	82.9 (22.8-191.7)
P value		0.70	0.04	0.0001	0.0001
PCT(ng/ml)	Sepsis	1.2 (0.32-3.1)	2.8 (1.5-4.9)	19 (10.7-92.7)	94.7 (25.7-208)
	Non-sepsis	0.9 (0.38-1.9)	2.4 (0.6-4.0)	2.9 (1.0-6.4)	11.03 (5.9-20.7)
P value		0.072	0.070	0.0001	0.0001
HMGB1(ng/ml)	Sepsis	17.6 8.9(26.9)	9.8 (5.9-19.8)	10.2 (5.9-24.9)	6.0 (1.7-13.6)
	Non-sepsis	15.4 (7.9-20.9)	8.9 (2.8-16.9)	7.7 (1.7-15.7)	4.9 (1.9-9.8)
P value		0.22	0.09	0.007	0.046

Table.3 Comparison of serum cytokines level between sepsis with survival and death patients.

Variables(pg/ml)	Value for group{Median(Min-Max)}		
	Healthy controls(n=50)	Sepsis with Death (n=16)	Sepsis with survival (n=9)
TNF-α			
Day 0		240.6(98.7-421.2)[a,c]	780.6(578.8-986.5)[b]
Day 3	32.1(27.4-64.7)	509.3(115.3-847)[a]	267.9(115.3-463.8)[b]
Day 7		352(115-780.6)[a]	297.6(152.6-528.8)[b]
Day 14		386.5(61.4-528.8)[a,c]	103.7(16.1-165.9)[b]
IL-6			
Day 0		136.3(72.6-189)[a,c]	75.7(65.7-86.6)[b]
Day 3	45.6(38.6-154.8)	138.4(90.8-210.9)[a,c]	84.2(74.4-98.7)[b]
Day 7		184.1(136.8-405)[a,c]	124.8(89.6-151.5)[b]
Day 14		332.6(210.9-958.4)[a,c]	137.9(98.7-156.3)[b]
IL-10			
Day 0		559.2(254.3-1139)[a,c]	82(35.3-366.9)[b]
Day 3	32.3(24.6-86.5)	258.4(206.5-794.2)[a,c]	147.3(25.6-306.5)[b]
Day 7		250(138.2-535.4)[a]	198.1(95.9-387.6)[b]
Day 14		156.3(45-366.8)[a]	216.8(18.2-606)[b]
MCP-1			
Day 0		24.6(3.9-32.2)	16(7.1-37.7)
Day 3	19.4(16.2-57.6)	97.6(23.4-131.8)[a]	94(26-158.5)[b]
Day 7		109.3(71.1-232.3)[a,c]	36.4-(22.1-93.1)[b]
Day 14		106.6(69.3-194.8)[a]	93.9(55-146)[b]
TGF-β			
Day 0		24.6(3.9-55)[a]	21.9(5.9-49.1)[b]
Day 3	10.5(6.4-18.9)	46.6(22.1-203.1)[a]	89.1(22.6-155)[b]
Day 7		90.3(22.1-232.3)[a]	99.6(26.6-194.8)[b]
Day 14		108(55-153.1)[a]	106.6(69.3-194.8)[b]
CRP(mg/L)			
Day 0		43(21.2-97.5)[a]	73.8(21-95.8)[b]
Day 3	4.7(2.9-13.8)	92.8(83.8-122.6)[a]	80.8(28.7-141.7)[b]
Day 7		189.6(165.9-234)[a]	192.2(102.8-222.7)[b]
Day 14		192.8(187.9-265.8)[a]	214.4(178.6-256.3)[b]
PCT(ng/ml)			
Day 0		1.0(0.49-3.1)	0.72(0.32-2.9)
Day 3	0.72(0.07-1.2)	3.4(1.5-4.9)[a,c]	1.9(1.7-3.0)[b]
Day 7		41.7(11.0-92.7)[a,c]	13.8(10.7-32.9)[b]
Day 14		171.8(72.9-208)[a,c]	87.9(25.8-97.8)[b]
HMGB-1(ng/ml)			
Day 0		18.9(8.9-26.9)[a]	13.9(10.7-20.6)[b]
Day 3	1.2(0.26-2.2)	9.6(6.0-19.8)[a]	10.6(5.9-13.6)[b]
Day 7		10.6(6.2-24.9)[a]	12.0(5.9-15.07)[b]
Day 14		6.3(1.7-13.6)[a]	5.3(3.9-9.2)[b]

Table S1, Comparison of serum cytokines levels between hemorrhagic shock patients who developed sepsis with other didn't.

Cytokine (pg/ml)		Day 0 Median (Range) (n1:25,n2:71)	Day 3 Median (Range) (n1:25,n2:71)	Day 7 Median (Range) (n1:25,n2:71)	Day 14 Median (Range) (n1:25,n2:71)
IL-1β	Sepsis	812.6 (215.3-1861.3)	497.3 (168.6-1745.3)	429.3 (77.3-1572.3)	114.3 (7.3-1793.4)
	Non-sepsis	723.3 (168.6-1861.3)	482.6 (69.4-1031)	264 (51-879.3)	133.3 (16-812.6)
P value		0.94	0.48	0.10	0.96
IL-12	Sepsis	11.3 (15.6-285.2)	36.5 (2.6-126.9)	43.4 (7.1-126.9)	37 (15.6-113)
	Non-sepsis	11.3 (5.3-380.8)	69 (4.3-273)	55.6 (8.6-187.8)	53 (14.3-161.7)
P value		0.91	0.30	0.98	0.09
IFN-γ	Sepsis	94 (54.9-201.8)	75.3 (25.3-117)	66.5 (18-100.9)	47.8 (11.4-74.3)
	Non-sepsis	94 (41.6-201.8)	75.4 (41.6-109)	65 (13.6-98.3)	41.6 (13.6-94)
P value		0.25	0.74	0.32	0.70
TNF-α	Sepsis	335.5 (67.6-1415.8)	320 (16-847)	275.8 (42.3-780.5)	302.6 (16.1-528.8)
	Non-sepsis	525.8 (104.7-986.4)	227.6 (16.3-772.9)	140.7 (39.4-671)	84.7 (22.6-181.4)
P value		0.015	0.06	0.007	0.001
IL-2	Sepsis	24.5 (4.0-56)	23.5 (12-58.7)	34.3 (10.4-77.5)	40.6 (12.5-210)
	Non-sepsis	20 (4-56)	24 (8-62.4)	31.5 (8.31-85)	34.3 (15.6-154)
P value		0.51	0.75	0.85	0.85
IL-4	Sepsis	24.9 (1.5-89.5)	31.7 (6.3-135.9)	55 (13.6-194.8)	109.4 (45.8-194.8)
	Non-sepsis	23.4 (3.6-119.6)	33.5 (14.6-101.5)	33.4 (22.1-153)	106.5 (23.4-153)
P value		0.97	0.78	0.58	0.21
IL-6	Sepsis	287.81 (50.2-539.3)	315 (74.4-1054.3)	139.3 (89.6-954.8)	367.2 (98.7-958.3)
	Non-sepsis	88 (33.2-582)	90 (70.2-1035)	138.2 (50.2-1024.7)	139.3 (101-554.5)
P value		0.02	0.01	0.42	0.04
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	Non-sepsis	56.5 (15.1-104.8)	63.9 (25.5-101.6)	74.8 (22.8-104.8)	82.9 (22.8-191.7)
P value		0.70	0.04	0.0001	0.0001
PCT(ng/ml)	Sepsis	1.2 (0.32-3.1)	2.8 (1.5-4.9)	19 (10.7-92.7)	94.7 (25.7-208)
	Non-sepsis	0.9 (0.38-1.9)	2.4 (0.6-4.0)	2.9 (1.0-6.4)	11.03 (5.9-20.7)
P value		0.072	0.070	0.0001	0.0001
HMGB1(ng/ml)	Sepsis	17.6 8.9(26.9)	9.8 (5.9-19.8)	10.2 (5.9-24.9)	6.0 (1.7-13.6)
	Non-sepsis	15.4 (7.9-20.9)	8.9 (2.8-16.9)	7.7 (1.7-15.7)	4.9 (1.9-9.8)
P value		0.22	0.09	0.007	0.046

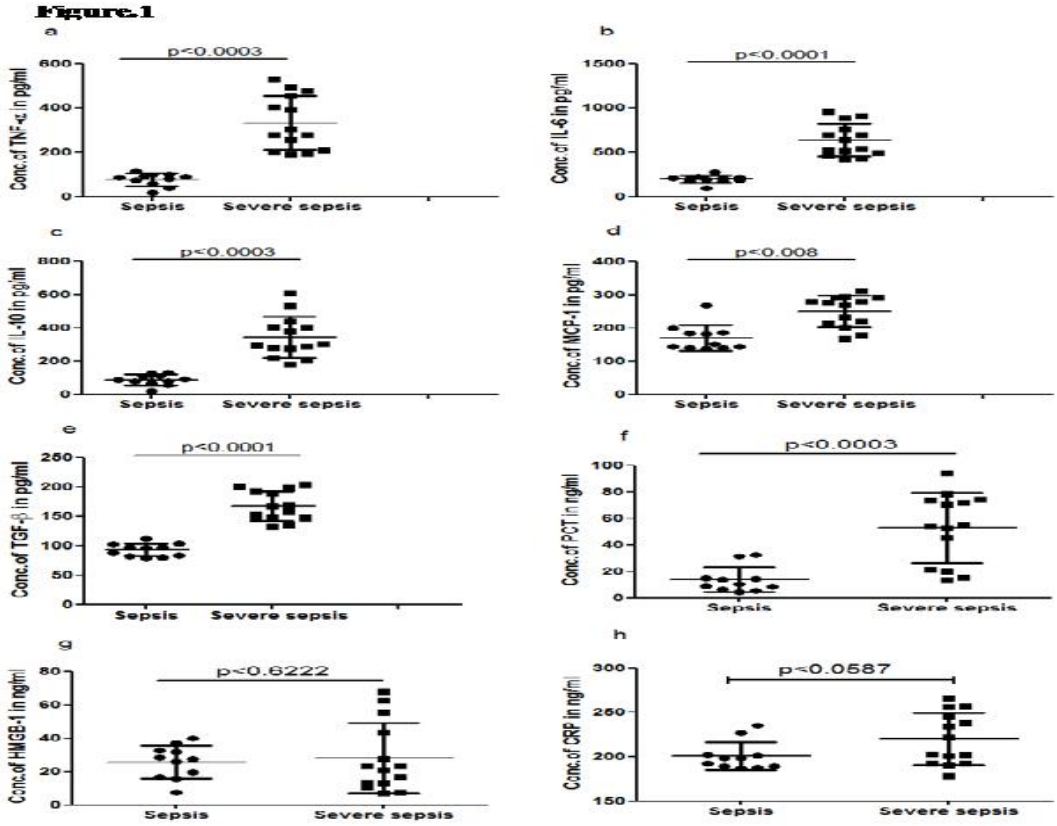

Figure 1. Serum cytokine levels in sepsis (n=11) and severe sepsis (n=14) patients were measured on day 14 after injury. Figures 1a, b, c, d, e, f, g and h showed the median and inter quartile range of cytokines TNF-α, IL-6, IL-10, MCP-1, TGF-β, PCT, HMGB1 and CRP respectively. Pairs were compared using the Mann-Whitney U test. The *P* value of the differences between pairs indicated by $p<0.05$.

Figure.2

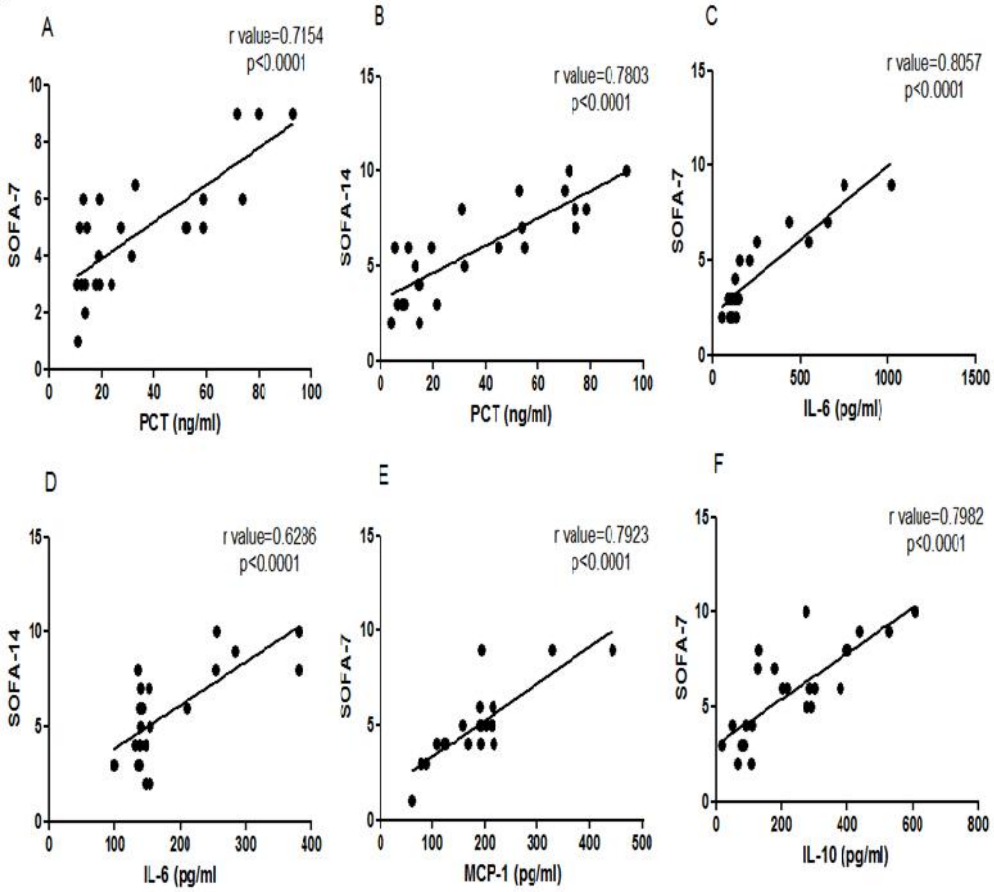

Figure 2. Pearson coefficient of correlation between the serum levels of PCT, IL-6, MCP-1 and IL-10 with SOFA score on days 7 and 14 were calculated in sepsis patients by graph pad prism software.

3. Discussion:

In this study, we have evaluated the immune response in patients with hemorrhagic shock who suffered from trauma, with or without sepsis and assessed the prognostic values of pro-inflammatory and anti-inflammatory cytokines, MCP-1, CRP, HMGB-1 and PCT regarding severity and outcomes. When we compared the cytokine profiles of hemorrhagic shock patients who developed sepsis with those of others who didn't develop sepsis, a significant imbalance in the levels of pro-inflammatory (TNF-α, IL-6), anti-inflammatory cytokines (IL-10), MCP-1, CRP, PCT and HMGB1 were observed.

We observed those patients who had low serum level of TNF-α at day 0, but elevated at other time points had higher risk of developing sepsis complications in the ICU and subsequently died. However, those patients who had elevated levels of TNF-α at day 0 and decreased on subsequent time intervals recovered without any complications.

Patients with poor outcomes due to sepsis had persistently elevated level of TNF-α and IL-6 while patients with favourable outcome showed subsequent reduction in serum levels of these cytokines. Serum level of IL-6 showed the positive correlation with SOFA score in those hemorrhagic shock patients who had developed sepsis while serum level of TNF-α didn't showed correlation with SOFA score.

Patients who had persistently elevated levels of IL-10 on various time intervals developed severe sepsis and they succumbed to the illness. Our study showed that those patients who had elevated level of IL-10 at day 0 had more chances of developing sepsis in the ICU. It also showed positive correlation with SOFA score in the ICU. Csontos et al. (2010) demonstrated higher level of IL-10 as a poor prognostic marker in patients with burn with sepsis [11] . Lorenta et al. (2009) showed that serum IL-10 levels were higher and level of TNF-α were lower in patients who died from sepsis in comparison to surviving patients [12]. Frink et al. (2009) reported high plasma levels of IL- 10 in patients with multiple organ dysfunctions (MODS). The work of Marchan et al. has been supported by our finding [13]. He reported that up regulation of plasma level of Interleukin-10 up- peaked during the first 24 hrs and decreased progressively till the 5th day in septic shock patients and positive correlation with SOFA score on 7th day in the ICU.

The diagnostic and prognostic value of serum procalcitonin, C-reactive protein, TNF–α and IL–10 in 39 patients with community acquired sepsis, severe sepsis and septic shock was investigated by [14]. In this trial, he reported those patients who had severe sepsis (septic shock) and died due to sepsis had significantly high level of serum procalcitonin during first 72 hours. Insistently high procalcitonin levels for 72 hours are regarded as an indicator for poor prognosis. In this trial, he observed C-reactive protein level did not show any significant between the groups and was reported to have no value as an indicator for prognosis. Serum level of TNF–α was found to be elevated in death patients during early stage (first 24 hours), but did not show significant difference between sepsis and severe sepsis groups. IL–10 level was reported to be significantly high in patients with severe sepsis and in patients who died during all measurements. But in our study, serum level of procalcitonin was found significantly elevated at all time points in patients who had sepsis and also elevated with severity of disease. Further, we observed sepsis with death patients have high levels of procalcitonin and showed positive correlation with SOFA score in the ICU. Likewise, serum levels of CRP was observed elevated in sepsis patients, but did not show any significant difference between the groups and was reported to have poor prognostic value. Against to his report, our study showed serum levels of TNF-α and IL-10 is good prognostic value for sepsis and IL-10 positive correlation with SOFA score in ICU. Özbalkan et al. (2004) reported that IL-10 peak levels in surviving patients without sepsis had lower levels than patients who died [15]. Gogos et al.(2009) also reported significant high levels of IL – 10 and TNF–α in patient who died from sepsis [16].

It is increasingly recognized that the inflammatory response and deregulated cytokine production plays key roles in the development of multiple organ dysfunction [17]. Clinically defined, early sequential analysis of organic dysfunction in severe sepsis has proven to be a good predictor of outcome [18]. However, the cytokine patterns associated with the evolution of organ dysfunction are not well established. A more restricted panel of cytokines and other markers (only four out of 13) namely PCT, IL-6, IL-10 and MCP-1 were found to correlate positively with organ dysfunction, as assessed by the SOFA score on days 7 and 14. Of these four cytokines IL-10 and PCT exhibited the best performance .Chao et al. have been reported in rat model of sepsis that serum level of HMGB-1 is highly correlating with severity [19]. However, our finding support the elevation of serum level of HMGB-1 in post-hemorrhagic sepsis patients, but it did not showed any positive correlation with severity of sepsis and outcomes of patients.

Limitations and future directions: The serum levels of cytokines measured at different time points and compared to severity scales may not reflect disease activity within the cell. The development of diagnostic tools that allow real- time and accurate determination of biomarkers concentration may significantly improve the characterization of individual inflammatory state of the patient. All the scoring system used to measure the multiple organ failure (MOF) has some limitations [20]. The results of our analysis need to be confirmed in a large-scale prospective study and should also be supported by data from other ethnic populations, with the hope that this information can be used to develop novel, perhaps individual-tailored, anti-inflammatory therapies for the prevention of sepsis development after major trauma.

4. Conclusion:

In conclusion, our study showed that patients with trauma hemorrhagic shock had a significant risk of the development of MOF and/or sepsis and severe sepsis (septic shock) and calamitous outcomes. Possible indices therefore include secondary deterioration of organ function or ongoing signs of sepsis accompanied

by a secondary rise of pro-inflammatory cytokines. Consequently, in addition to the initial evaluation of the risk profile of each patient based on the ISS, S.I, and age, immunological monitoring of serum cytokine biomarkers, coupled with daily evaluation of the organ function, is recommended to identify the patients at risk of clinical deterioration. It is our assumption that treatment strategies should be focussing on the early detection of inflammatory mediators and deterioration of organ function before the clinical appearance of MOF. Furthermore, investigation is necessary to improve the detection systems with respect to the scoring systems used and the application of serum biomarkers to predict outcome. Early identification of patients at high risk of death and disability associated with sepsis could greatly facilitate the development of newer and effective treatment interventions for trauma patients.

In the same way, future studies at bedside can be used to examine whether various therapeutic strategies can attenuate cytokine production and improve outcomes or, at the very least, whether these cytokine markers can be used to predict mortality in trauma haemorrhage patients.

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