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Clinical significance and Prognostic Role of Serum Tenascin-C in SepticPatients in Intensive Care Unit

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Abstract

Background: Sepsis exists on a continuum of severity ranging from infection to septic shock, which can lead to multiple organ dysfunction syndrome (MODS) and death.Tenascins are a group of extracellular matrix glycoproteins that are expressed during the development of multicellular organisms. Tenascin-C may be an inflammation promoting factor for sepsis and its expression may be increased in various tissues.

Aim of the study: The aim of this study wasto investigate the relationship between serum Tenascin- C levels and disease severity and progression in patients with sepsis at ICU.

Patients and methods: This was a case control study that involved total number of 39 patients 9 apparently healthy subjects, 20 patients with sepsis in ICU and 10 diseased patientswithout sepsis in ICU.All participants were subjected to full clinical examination and laboratory investigations including CBC, CRP, blood culture, and Tenascin C was measuredby ELISA for all participants.

Results: There was no significant difference between all studied groups as regard age or sex.There were high significant rise in CRP, PCT, TLC, as well as Tn-C in septic patients in relation to that of non-septic patients.Hemoglobin concentration showed significant decrease in septic patients than that in non-septic patients.There were 12 cases diseased with sepsis had positive blood cultures.tenascin C level was higher among non-survivor septic patients than survivors indicating its association with the severity and prognosis of sepsis.

Conclusion: Tn-C may be involved in the pathogenesis of sepsis and serve as a potential biomarker and therapeutic target as the sensitivity of tenascin-c to predict sepsis cases vs control was 75%, specificity was 100%.

Keywords: Sepsis, Tenascin-C (TN-C), Intensive Care Unit (ICU).

1. Introduction:

Sepsis is a systemic inflammatory response caused by infection and has a high risk of multiple organ dysfunction syndrome (MODS) and death. Sepsis was defined as the presence of microbiologically confirmed infections, acute organ failure, and systemic inflammatory response syndrome (at least two of the following parameters: (1) temperature>38 °C or<36 °C; (2) heart rate>90 beats / min; (3) Respiratory rate>20 beats/min or PaCO2 < 32 mmHg; (4) white blood cell (WBC) count >12,000 or<4000 cells/mm3, or>10% immature form) **(1)**.

It is currently believed that severe sepsis causes systemic inflammatory response syndrome (SIRS) and has a compensatory anti-inflammatory response. At the same time, the complex interaction

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between pro-inflammatory and anti-inflammatory responses determines the outcome of sepsis. Therefore, new biomarkers are required for early diagnosis and prognosis prediction of severe sepsis patients **(2)**.

Tenascins are a group of extracellular matrix glycoproteins that are expressed during the development of multicellular organisms, and are involved in a variety of pathological processes such as inflammation, tissue damage, tumor angiogenesis and metastasis. Tenascin-C is a multi-domain protein linked by disulfide bonds. Obvious expression of tenascin-C can be measured during embryonic development, especially in sites with higher cell turnover such as stem cell niches. The expression of tenascin-C in adults is limited to the site of tissue injury, usually temporary, and the expression level of tenascin-C returns to normal after tissue repair is completed. In contrast, the sustained high expression of tenascin-C is common in inflammation, tissue remodeling, and autoimmune diseases **(3)**.

Infection and injury can induce the expression of tenascin-C, which promotes the body to produce an effective immune response to bacterial lipopolysaccharide (LPS). Tenascin-C enhances the synthesis of pro-inflammatory cytokines in macrophages that is activated by LPS through toll-like receptor 4 (TLR4), while inhibiting the synthesis of anti-inflammatory cytokines. Therefore, tenascin-C plays a role in regulating the axis of inflammation in LPS-activated TLR signaling **(4).** We aimed in this study to to investigate the relationship between serum Tenascin- C levels and disease severity and progression in patients with sepsis at ICU.

2. Patients and Methods:

2.1. The current study was conducted asacase control study.A total number of 39participants were included in the study. They were collected from intensive care unit (ICU) in Zagazig University Hospitals during the period from March 2019 till November 2019 after obtaining the approval of the institutional review board (IRB) of Zagazig University.The selected participants were classified into three groups: **Group 1** included 9 subjects apparently healthy: 5 males and 4 females aging from 29 to 67 years old serving as control group.**Group 2** included 20 patients with sepsis in ICU: 14 males and 6 females aging from 21 to 71 years old;**Group 3** included 10 diseased patients without sepsis in ICU: 7 males 3 females aging from 45 to 69 years old.Assuming that mean ±SD of serum tenascin-c in sepsis patients VS control groups was mean 55±17 VS mean 40±15. The sample of study was calculated to be 39 patients using openEpi with power of test 80% and CI 95%.

2.2.A consent form approved by the committee of human rights in research in Zagazig University was obtained from each participant before the study initiation.

2.3. Patients who were included in this study of age more than 18 years and have sepsis diagnosed by laboratory investigations and clinical presentation.

2.4. All patients who having fever, increase TLC fornon-septic cause, tumor or autoimmune disease were excluded from the studywere excluded from the study.

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2.5. The patients who met the inclusion criteria and were suitable candidates for the study have been subjected to: Full clinical assessment which includes complete history taking, clinical examination and SOFA score at time of admission. Routine laboratory testing including: -CRP, Procalcitonin, Complete blood count (CBC), Blood cultures and sensitivity tests.

2.5. Specimen collection and storage:

Three ml of venous blood by vein puncture were collected under complete asepticcondition from all subject in a sterile separator gel tube for serum isolation and left toclot. Centrifugation done for 20-min at the speed of 2000 -3000 r.p.m and supernatant removed and stored at (- 80) ℃ till analysis.

2.6. Measurements of Tenascin – C:

Tenascin-C was measured in serum samples by ELISA. Kit was provided fromSunRed biotechnology company (China) Catalogue No. 201-12-1415 named Humantenascin-C (TN-c) ELISA Kit.

All samples were measured in duplication

2.7. Statistical analysis:

The data was analyzed using SPSS 19.0 statisticalsoftware. Continuous variables were expressed as median (quartile), and categoricalvariables were expressed in frequency (percentage).Wilcoxon-Mann-Whitney testwas performed to compare continuous variables between survivors and nonsurvivors,including age, SOFA scores, ICU time, CBC, CRP, Procalcitonine,Blood culture&sensitivity . Chi-square test was performed to compare categoricalvariables between survivors and nonsurvivors, including gender, site of infection, thepresence of mechanical ventilation and septic shock. Spearman's rank sum test wasperformed to analyze the correlations between tenascin-C and age, SOFA scores, ICU time, WBC, CRP.

3. Results:

Regarding mean ± SD for age in healthy group was 44.44 ± 12.29, with range of (29.0 – 67.0), mean ± SD for age in group with sepsis was 51.55 ± 18.29, with range of (21.0 – 71.0), and mean ± SD for age in diseased group without sepsis was 58.0 ± 7.77, with range of (45.0 – 69.0).

In group with sepsis 60 % were males compared to 70% in diseased group without sepsis and 66.7 % in control group. There is no significant difference between all studied groups as regard age or sex as they were selected from the start **(Table 1).**

There were high significant rise in CRP, PCT as well as TLC in septic patients in relation to that of non-septic patients while hemoglobin level shows significant decrease in septic patients than that in non-septic patients. As regard platelets there is no significant difference between both groups**(Fig. 1, 2,3,4).**

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There were 12 cases diseased with sepsis had positive blood cultures. Three cases were positive for gram negative organisms as Klebsiella pneumonia, Acinetobacter baumannii and E.coli. Nine cases were positive for gram positive organisms as: Staph haemolyticus, Staph epidermis and Staph hominis**(Table 2).**

.There were of sepsis cases 14 cases died by a percent of 70%, 3 discharged by a percent of 15% and 3 still in ICU by a percent of 15%. Mean SOFA score was 6.75±2.97.In regard to patients without sepsis who were admitted to ICU due to different causes, 4 of them had died representing 40 % of their number**(Fig.5).**

There was significant rise in Tenascin-C level among patients with sepsis when compared with healthy and among patients with sepsis when compared with patients without sepsis, while no significant difference was obtained between non septic patients and healthy control (Table 3).

Sensitivity of tenascin to predict cases with sepsis vs control was 75%, specificity was 100%, PPV was 95% and NPV was 70% at a cut off >8100 **(Table 4).**

Table (1): Comparison between the three studied groups as regard demographic data

	Healthy control (n = 9)		**Diseased with sepsis (n = 20)**		**Diseased without sepsis(n = 10)**		**Test of Sig.**	**p**
	No.	**%**	**No.**	**%**	**No.**	**%**		
Sex								
Male	6	66.7	12	60.0	7	70.0	χ^2=	^{MC}p=
Female	3	33.3	8	40.0	3	30.0	0.380	0.380
Age								
Range	29.0 – 67.0		21.0 – 71.0		45.0 – 69.0		F=	0.159
Mean ± SD.	44.44 ± 12.29		51.55 ± 18.29		58.0 ± 7.77		1.933	

Table (2): Percentage of positivity of blood culture results among studied groups.

Blood Culture	Diseased with sepsis (n = 20)		Diseased without sepsis (n = 10)		χ^2	^{MC}p
	No.	**%**	**No.**	**%**		
Negative	8	40.0	10	100.0	16.271*	<0.001*
Positive	**12**	**60.0**	**0**	**0.0**		
Gram –ve	3	15	–	–	–	–
Gram +ve	9	45	–	–	–	–

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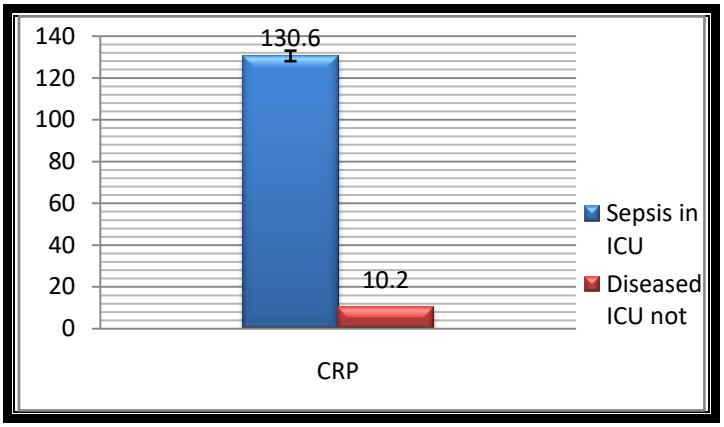

Figure (1): Mean ± SD CRP between the studied groups.

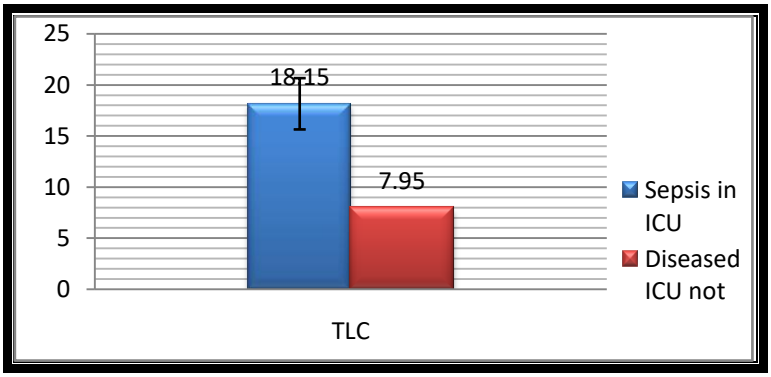

Figure (2): Mean ± SD TLC between the studied groups.

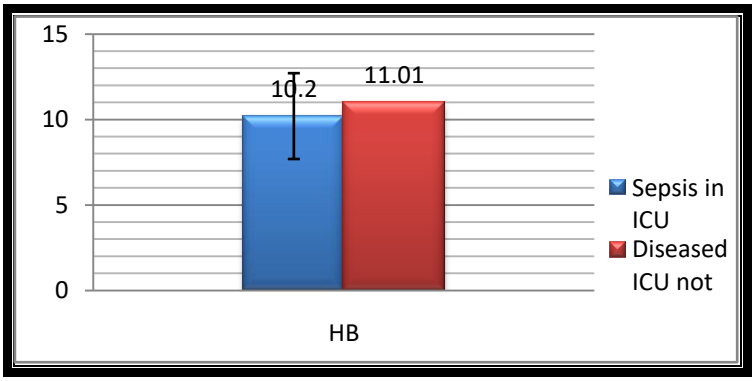

Figure (3): Mean ± SD HB between the studied groups.

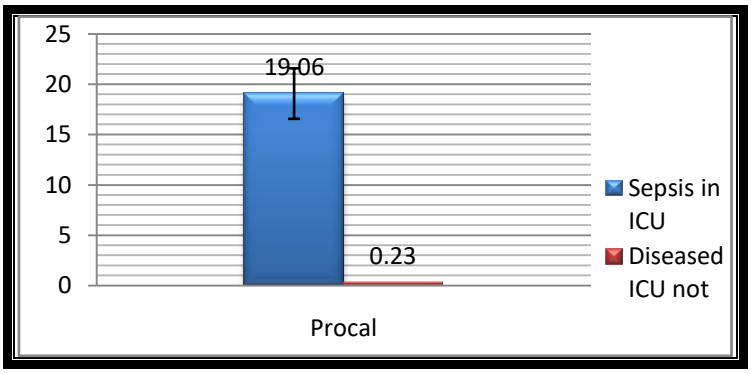

Figure (4): Mean ± SDPCT between the studied groups.

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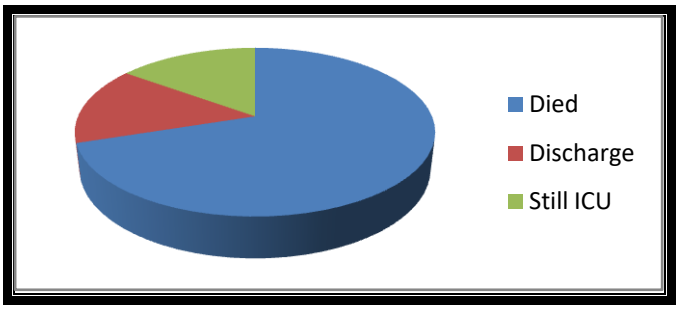

Figure (5): Prognosis of the studied cases.

Table (3): Comparison between the three studied groups according to TN-c level.

Tc	Healthy control (n = 9)	Diseased with Sepsis (n = 20)	Diseased without sepsis (n = 10)	H	P
Range ng/l	950.0 – 12000.0	2000.0 – 82000.0	464.0 – 50000.0	8.522*	0.014*
Mean ± SD. ng/l	4906.7± 3743.2	19430.0 ±19249.0	11916.4 ±14825.9		
IQR	1410.0 – 7000.0	8000.0 – 22350.0	2800.0 – 18000.0		
Sig. bet. Groups.	p_1=0.002*,p_2=0.005*,p_3=0.280				

Table (4): Agreement (sensitivity, specificity) for Tn-C to predict sepsis cases vs control.

	AUC	95%CI	P value	Cutoff	Sensitivity	Specificity	PPV	NPV
Tc	**0.878**	**0.756-0.999**	**0.001**	**>8100**	75.0	100.0	95.0	70.0

4. Discussion:

The laboratory indicators used for the clinical diagnosis of sepsis include CRP , IL6 IL 8, TNF α and PCT none of which are ideal diagnostic indicators due to their differences in diagnostic specificity and sensitivity **(5).**Therefore, it is important to find new diagnostic indicator to improve the treatment of sepsis and reduce the mortality **(6).**
Diagnosis of sepsis is often made empirically at the bedside upon presentation, or retrospectively when follow-up data returns (e.g., positive blood cultures) or a response to antibiotics is evident. Importantly, the identification of a culprit organism, although preferred, is not always feasible since in many patients no organism is ever identified. In some patients this may be because they have been partially treated with antibiotics before cultures are obtained **(7)**.

The expression of Tenascin c in adults is limited to the site of tissue injury and usually temporary and the expression level of Tn-c returns to normal after tissue repair is completed. In contrast, the sustained high expression of TN-c is common in inflammation, tissue remodeling and autoimmune

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disease **(8).**

Tn-C is involved in the pathogenesis of sepsis by many mechanisms, and it is a broad inducer of inflammation **(9).**

So this studywas designed to investigate the relationship between serum Tn-C levels and disease sensitivity and prognosis in patients with sepsis.

In this study, the selected participants were classified into 3 groups: group 1 included 9 healthy controls: 5 males and 4 females aging from 29 to 67 years old; group 2 included 20 sepsis patients in ICU : 14 males and 6 females aging from 21 to 71 years old ; group 3 included 10 patients diseased in ICU not having sepsis: 7 males 3 females aging from 45 to 69 years old. There were no significance difference among studied groups as regard age and sex.

The results of the present study were in agreement with that of **(10),(11)** as well as the results of **(12).** All this studies provide the evidence that the occurrence of sepsis is not associated with certain age group, but relies on other factors.

CRP is a biomarker involved in more than one inflammatory cascade amplification, now it is applied to sepsis diagnosis **(11)**

The result of this study revealed that there was statistical significant increase in CRP level in septic patients when compared with non-septic patients ($p < .001$). this result was in accordance with that of **(13)** who reported that CRP exhibited a higher level in serum of sepsis group than the non-septic patients in ICU. The same results were obtained by **(14).**

The results of (**6)** disagrees with our result where they reported that the level of CRP in sepsis group and non-sepsis group were significantly higher than this of control group but, there was no significant difference between non sepsis group and sepsis group.

Procalcitonin is a prohormone of calcitonin. Under normal condition, it is only produced by c-cells of thr thyroid gland. During infection many organs can produce PCT in response to proinflammatory cytokines e.g: TNF, IL 6. It shows an early increase during infection that is why it is used as infection biomarker **(15).**

It is used as a laboratory diagnostic indicator of sepsis, but its value for diagnosis, prognosis and the differential diagnosis with non-septic patients is not satisfactory **(16)** but can be used to evaluate severity of sepsis **(13)**

The present study revealed an elevated level in PCT among sepsis group when compared with non-septic group. This result came in accordance with that of **Su et al (13)** and **Zhao et al (6).** The recent study of **Tambo et al (17)** found that elevated PCT before treatment might predict the development of sepsis in patients with obstructive acute pyelonephritis.

The current study revealed a significant rise in WBCs in sepsis than in non-sepsis patients. This result agrees with that of **Czupryna et al (18)** who reported that mean WBCs count was significantly higher in severe sepsis.

Zhao et al (6) reported that there is no significant difference in CRP and WBCS in sepsis than non-sepsis.

As regard haemoglobin concentration, it was 11.01 g/dl in septic patients and it was significantly lower in patients with severe sepsis. This result was in agreement with **Czupryna et al (18) &Ekregbesi et al (19)** who said that in human sepsis Hb concentration decrease in hepcidindependant manner during admission. So, anaemia in patients with severe sepsis may be caused either by haemolysis or by inflammatory process.

The identification of causative organism through blood culture is still the gold standard of

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diagnosis of sepsis. However, confirmation of pathogen by cultures is slow and is often yield false negative result **(20).**
In the present study, 60 % of examined patients (12/20) with sepsis the blood cultures were positive. In both groups, gram positive bacteria dominated with 45 % (9/12) e.g: staph haemoliticus, staph epidermidis and staph hominis.

This result agrees with that of **Czupryna et al (18)** who reported that 55.1 % of their patients were positive blood cultures, but with staph. aureus as the most common pathogen.

The gram-negative organisms as klebsiellapneumonia,acinetobacterbaumannii and E. coli represent 15% of positive blood cultures in sepsis (3/12). The same result was reported by **Esteban et al (21)** but they showed that gram negative bacteria were dominant with E. coli as the common pathogen.

In the present study (14/20) 70 % died and (6/20) 30 % survived, 15 % discharged from ICU and 15 % stayed in ICU. The mean of SOFA score was 6.75 ± 2.97.
The result of **Czupryna et al (18)** reported that SOFA score mean was 2 while in sepsis group the score ranged from 0-9 points in severe sepsis. The same results were obtained by **Su et al (13)** who stated that SOFA score was of diagnostic value for sepsis severity.
Yuan et al (14)hypothesize that in sepsis lipopolysaccharides drives expression of Tenascin –c in injured tissue include extracelluar matrix which is released into circulation, therefore change in tenascin- C may reflect the extent of tissue damage, disease severity and prognosis.
In this study, the tenascin – c level revealed that mean ± SD in healthy control was 4906.7 ±3743ng/l, which is highly statistical significant rise in septic patients when compared with healthy control. The septic patients had higher rise in level of Tenascin –c than non-septic patients, while there is no significant difference between non septic patients and healthy control.

These results agree with that of **Yuan et al (14)**who reported that in septic patients serum Tenascin – c levels were significantly higher in non-survivor compared to survivors. Also, with further analysis the tenascin-c level showed association with clinical severity, inflammatory mediators of patients and acted as an independent prognostic factor.
There was a significant difference between survivor and non-survivor septic patients as regard tenascin level as it was higher in nonsurvivor septic patients than that in survivors (p value 0.041) .

As regard correlation between tenasin –c level and the other data of the present study, there is no statistically significant correlation with any of the studied parameters either in septic or non-septic groups.
This result agreed with that of **Meijer et al (22)** while the **Yuan et al (14)**reported that in patients with sepsis serum tenascin c levels were significantly positively correlated with SOFA score (p=.011) serum creatinie (p=.006) and CRP (p=.001).
To evaluate sensitivity and specificity of serum tenascin – c we did ROC curve. The values obtained in this study were:
AUC for Tenascin-c for distinction between septic and non-septic patients was 0.865 (95 % CI 0.73-0.99) cut off at >9000 ng/dl at which Tn-C had specificity of 75% and sensitivity of 100% ppv 95 NPV 42, 1.

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AUC for Tenascin-c for distinction between septic and control groups was 0.878 (95% CI was 0.756 - 0.999) at a cut off > 8100 at which Tn-C had sensitivity of 75%, specificity of 100%, PPV was 95% and NPV was 70%.

This result agrees with that of **Yuan et al (14)** who said that ROC curve is used to determine the cut-off point of serum tenascin-C that distinguishes between the survivors and non survivors. The high serum Tn-C is closely associated with high mortality in septic patients.

Meijer et al (22) studied the level of Tn-C among patients who had pneumonia and other healthy by using the ROC curve. They concluded that, with AUC of 0.67, plasma Tn-C did not adequately distinguish CAP (community acquired pneumonia) from no-CAP patients.

5. Conclusion:

We concluded from this study that the concentration of serum Tn-C in septic patients may aid inearly sepsis diagnosis and severity assessment. Serum levels of Tn-C are associated with clinical severity, systemic inflammatory response, and 30-day mortality.

Our results suggest that Tn-C may be involved in the pathogenesis of sepsis and serve as a potential biomarker and therapeutic target as the sensitivity of tenascin-c to predict sepsis cases vs control was 75%, specificity was 100%.Despite advances in diagnostics and treatment, sepsis in still a major medical problem with high mortality. Therefore, patients with severe sepsis should be treated in ICU setting even if no respiratory or circulatory failure is present in trial to decrease the mortality rate among sepsis patients.

6. ConflictofInterest: Noconflictofinterest.

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