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القيمة التخمينية للبروكالسيتونين, انترليوكين-١, انترليوكين -١٠, انترليوكين-١٣, وعامل نخر الورم-الفا في تشخيص انتان الدم

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الملخص

تهدف الدراسة الحالية إلى اختبار دور بعض المؤشرات في تشخيص إنتان الدم ضمن مرضى متلازمة الاستجابه الالتهابية الجهازية. شملت الدراسة ١٨٠ مريضا لديهم علامات متلازمة الاستجابه الالتهابيه الجهازية وخلال ٢٤ ساعة من دخولهم . تم ادخال المرضى لمستشفيات مختلفة في مدينة الموصل في الفترة من كانون الثاني ٢٠١٣ إلى كانون الثاني ٢٠١٤ . تم جمع عينتان من الدم من كل مريض, إحداهما لاستنبات الدم والعينه الأخرى فصلت عن طريق جهاز الطرد المركزي وحفظت مجمدة لعمل فحص ELIZA ل PCT, IL-1, IL-10,TNF-α و IL-13. إن PCT, IL-1, IL-10 و IL-13 لديهم فرق واضح بالمعدل بين حالات استنبات الدم الايجابية والسلبية. فحص PCT كان مرتفعا بصورة ملحوظة في حالات إنتان الدم $P=0.004$ مع حساسية عاليه (٩٣,٧%) وخصوصية (٩٠,١%). عدة مجموعات من العلامات استعملت في الدراسة الحالية لإيجاد أحسن مزيج من العلامات. نتيجة استعمال الحساسية والخصوصية الممزوجة ل PCT مع اثنان من السايتوكينات المضادة للالتهاب كان الأفضل (IL-10 و IL-13). وكانت علامات ممتازة بحساسية ١٠٠% وأعلى خصوصية.

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الكلمات الدالة:السايتوكينات المضادة للالتهابات, سايتوكينات, انتان الدم, متلازمة الاستجابه الجهازيه الاتهابيه.

Procalcitonin, Interleukin-1, Interleukin-10, Interleukin-13 and Tumor necrosis factor-alpha predictive value in the diagnosis of sepsis

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ABSTRACT

The present study is aimed to evaluate the role of some markers in the diagnosis of sepsis among SIRS (systemic inflammatory response syndrome) patients. The study included 180 patients with SIRS criteria within 24 hour of admission. They were admitted to different hospitals in Mosul city for the period from January 2013 to January 2014. From each patient two blood samples were collected, one for blood culture and the other separated by centrifugation and kept frozen for ELISA tests of PCT, IL-1, TNF-α, IL-10 and IL-13. IL-1, IL-10, IL-13 and PCT were having a significant difference in mean between culture positive and negative cases. PCT

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was significantly highly elevated in patients with sepsis (P=0.004) with high sensitivity (93.7%) and specificity (90.1%). Many combinations of markers were used in the current study to find out the best combination of markers. The results of using combined sensitivity and specificity of PCT together with 2 anti-inflammatory cytokines (IL-10 and IL-13) were the best. They were an excellent markers with 100% sensitivity and the highest specificity.

Keywords*: Anti-inflammatory cytokines, Cytokines, Sepsis, Systemic inflammatory response syndrome*

1. INTRODUCTION

Sepsis is a clinical term characterized by a marked attack upon the host by pro-inflammatory cytokines that has been precipitated by an infection. Importantly, sepsis represents the body's systemic response to severe infection. Not-uncommonly sepsis leads to one or more severe complications for example hypotension, cardiac failure, coma, renal failure, intravascular coagulation. This phenomenon termed multiple organ dysfunction [1,2]. It is important to differentiate patients with sepsis from those with SIRS, as treatments may be very different [3]. The mortality of patients with septic shock is high, 50-90 % [4]. Various attempts have been made to improve the diagnosis of infection. An alternative approach is the development of scores that combine several variables [5]. A recent prospective study supported the good predictive value of the IPS (Infection Probability Score) for a diagnosis of infection [6]. In sepsis an expanding number of cytokines have been found to be involved in the pathogenesis of the disease [7,8].

The use of a single marker can hardly provide the physician with the information needed. Instead, a set of markers reflecting both pro- and anti-inflammatory activation profiles that is simple enough to be provided by the

hospital laboratory as a 24 hour service would be ideal [4,9]. Various markers have been proposed over the years. Cytokine levels may seem an obvious choice as cytokines are key mediators of the inflammatory response to sepsis. Raised levels of certain cytokines have been well documented in patients with sepsis and some have been correlated with outcome [10]. However, no cytokine is specific for sepsis, and not all cytokine levels are raised at all-time points during the course of the disease. For example, tumor necrosis factor-α (TNF-α) levels are raised early in the course of sepsis, but raised levels are also found in other non septic conditions including as acute pancreatitis [11], and later in the disease process levels may fall.

2. MATERIALS AND METHODS

2.1 Patients: One hundred eighty critically ill patients who met the criteria of SIRS were enrolled in this study with in the first 24 hours of admission before or during the first 24 hours the administration of antibiotics.

All ages, both sexes were included and the patients were from different words: neonatal units, burn unit, emergency department, ICU and hospital words. The criteria for their classification were history investigations and clinical signs. SIRS criteria was defined as being the presence of more than one of four clinical criteria, Body temperature greater than 38ºC or less than 36ºC, Heart rate greater than 90 beats/ min, Respiratory rate greater than 20 breaths/ min or hyper-ventilation with a PaCO2 less than 32 mmHg, White blood cell count >12000/mm3, <4000/mm3, or with >10% immature neutrophils.

Exclusion criteria of patients on antibiotics for more than 24 hour. Patients with cancer were also excluded from the study. These patients were 103 (57.2%) males and 77 (42.8%) females. Their ages were between (1 day-75 years) and the mean age was 34.88±20.7. Fifty one patients (28.3%) were burn patients, whereas 46 were trauma patients. The remaining cases were patients with pancreatitis, pneumonia, empyema, mesenteric ischemia, postoperative patients, perforated vescus, and septic shock. The collection of samples was carried out from January 2013 to January 2014. The consent of agreement has been taken from the patient to take sample for the research. Agreement from the committee of medical ethics in Nineveh directory of health has also been taken.

2.2 Sample Collection: Two blood samples were collected from each patient, one for blood culture directly inoculated into sterile blood culture bottles containing brain heart infusion broth. About 5ml of blood for the second sample was separated by centrifugation and the serum divided into 5 labeled eppendrofes tubes and kept frozen at -20°C (one for each ELISA kit). For each patient double blood culture was done, in addition to ELISA tests.

2.3 Culture: The identification of blood culture was done using conventional culture media.

2.4 Biomarker determinationTwo Procalcitonin ELISA kit96 wells (MyBioSource Cat No. MBS265370), Two TNF-α ELISA kit96 wells (IBL international-Germany Cat No. BE55001), Two IL-1 ELISA kit96 wells (IBL international-Germany Cat No. BE51001), Two IL-10 ELISA kit96 wells (IBL international-Germany Cat No. BE53101), and Two IL-13 ELISA kit96 wells (IBL international-Germany Cat No. BE53131) were used and the procedure was done according to the manufacturer.

2.5 Statistical analysis: data were recorded on a specially designed questionnaire collected and entered Statistical Package for Social Sciences (SPSS) version 22 with the statistical

significance level of < 0.05. The results were presented as rates, ratios, frequencies, percentages in tables and figures. Chi square and T-test, sensitivity, specificity, positive predictive value and negative predictive value were performed to compare between both groups. The data were analyzed statistically by using tables, and bar charts.

1. RESULTS

IL-1, IL-10, IL-13 and PCT were having a significant difference in mean between culture positive and negative cases Table (1). TNF-α is a pro-inflammatory cytokine with a primary function to promote inflammation. In the current study the levels of TNF-α were statistically not significant in patients with sepsis.

Table (1) Different cytokines and culture positive and negative cases

Variables	Culture	No.	Mean (pg/ml)	p- value	t-test
IL1	Positive	79	4.7813	0.0001	Significant
	Negative	101	1.0759		
IL10	Positive	79	35.780	0.0003	Significant
	Negative	100	1.6784		
IL13	Positive	79	4.3454	0.0001	Significant
	Negative	101	1.4635		
TNF alpha	Positive	79	46.261	0.16	Non-significant
	Negative	101	34.776		
Procalcitonin	Positive	79	1012.6	0.004	Significant
	Negative	101	79.044		

Therefore, in patients with SIRS, there were a significant relation between culture positive cases (sepsis) and certain cytokines (IL-1, IL-10, IL-13 and

procalcitonin). The sensitivity and specificity of each cytokine were calculated and the most sensitive cytokine in culture positive cases was found to be the procalcitonin. PCT in the current study was proved to be a good indicator of sepsis with sensitivity 93.7% Table (2).Many combinations of markers were used in the current study to find out the best combination of markers (with higher sensitivity). The combined sensitivity and specificity tests of two markers are shown in Table 1.2. The highest sensitivity and specificity in combination of 2 markers is achieved by using PCT and IL-10 together.

Table (2) combined sensitivity and specificity tests for two cytokines

Test	Sensitivity%-Specificity%					
	PCT	**TNF-α**	**IL-1**	**IL-10**	**IL-13**	**CRP**
PCT	93.7-90.1 PCT alone	93.7-75.3	96.2-63.4	98.7-90.1	97.5-90.1	96.2-31.7
TNF-α		36.7-79.2 TNF-α alone	89.9-53.5	84.8-67.3	67.1-72.3	81.0-89.1
IL-1			77.2-76.2 IL1 alone	96.2-64.4	92.4-90.09	94.9-65.3
IL-10				74.7-91.1 IL10 alone	86.07-94	87.3-69.3
IL-13					60.8-89.1 IL-13 alone	89.9-84.2
CRP						58.2-48.2 CRP alone

Our goal to find out the ideal marker so in the second step we combined three cytokines. The results of using combined sensitivity and specificity of PCT together with 2 anti-inflammatory cytokines (IL-10 and IL-13) were excellent. They were found to be the excellent markers with 100% sensitivity and the highest specificity. The combined sensitivity and specificity of 3 markers were shown in Table (3).

Table (3) Combined sensitivity and specificity of PCT with 2 cytokines

Tests	Sensitivity %	Specificity %
PCT+TNF+IL-1	96.2	51.5
PCT+IL-1+IL-10	98.7	62.4
PCT+IL1+IL-13	98.7	58.4
PCT+TNF+IL-10	98.7	69.3
PCT+TNF+IL-13	97.46	78.2
PCT+IL-10+IL-13	100	90.1

The levels of the three markers (PCT, IL-10 and IL-13 were significantly higher in patients with sepsis than in patients without sepsis. Further, all three markers individually demonstrated significant *p*-values in sepsis patients. Remarkably, the performance of these markers was so strong as to render 100% sensitivity and 90.1% specificity.

3. DISCUSSION

The traditional approach to sepsis diagnosis was based on clinical signs and symptoms of sepsis, such as fever, tachycardia and tachypnoea, supported by relevant microbiological data (blood culture which usually takes 24 to 48 hours). More recently, biological laboratory markers (biomarkers) have been used, ranging from the relatively simple white blood cell count and C-reactive

protein (CRP) to more complex biomarkers, such as procalcitonin (PCT) or cytokine levels.

Interleukin-1 (IL-1) is one of several pro-inflammatory cytokines produced during infection. The sensitivity of IL-1 in the detection of cases with sepsis in the current study was 76% but it's of low specificity (55.5%). This is in agreement with other study in which higher serum levels of IL-1 compared to control was detected P<0.001 [12]. In a research the interest has been focused on IL-1 inhibition to improve outcome in sepsis and septic shock [13].

Interleukin-10 is an immunosuppressive mediator after injury or sepsis. The relation between IL-10 and culture positive in the present study was statistically significant. Two studies by José and co-authors in 2000 and Charalambos et al., 2000 showed that its levels were significantly higher in non survivors of sepsis so they considered that among various pro- and anti-inflammatory cytokines, IL-10 was more closely related to the severity of disease [14,15].

IL-13 is anti-inflammatory, its level in patients with sepsis in the current study were statistically significant. Its sensitivity is lower than IL-1 and in the present work. This result is in accordance with the result of other work by Collighan et al., 2004 in which the serum IL-13 concentration was significantly higher in the septic shock and IL-13 concentrations were higher in non-survivors [16].

A study by Fernando and co-authors demonstrated that the concentrations of IL-1, IL-10, IL-13 and TNF-α were significantly higher in septic shock patients than in those with severe sepsis. Cytokine concentrations were associated with severity and evolution of organ dysfunction [17].

In the current study the levels of TNF-α were statistically not significant in patients with sepsis. Its levels were elevated in most cases of inflammation not only septic patients so its sensitivity was low in the current study. This result is in accordance with other studies [11,18]. PCT in the current study was proved to be an excellent indicator of sepsis with sensitivity of 93.7%. This finding goes with Lacour et al., 2001 study where PCT showed a sensitivity of 93% and a specificity of 78% for detection of bacterial infection which is better than CRP which had a sensitivity of 89% and a specificity of 75% [19]. In addition, our result is consistent with other studies where PCT has been reported to differentiate between SIRS and sepsis [20], serve as a marker for sepsis in neonates [21] and to differentiate between fever of viral and bacterial etiology with more specificity than and similar sensitivity to CRP [22].

The short half-life (25–30 hours in plasma) of PCT, coupled with its virtual absence in health and specificity for bacterial infections, gives it a clear advantage over the other markers of bacterial infection. However, PCT is not a readily available diagnostic assay in most institutions. As a biomarker for bacterial infection, most studies find PCT to be a useful and accurate biomarker [23,24,25.26].

Several cytokines were assessed as potential markers in sepsis, but none of the cytokines studied has a sufficient specificity or sensitivity to be routinely employed in clinical practice. Several studies used combination of different markers. In these studies the bioscore had better predictive value for infection than any of the biomarkers alone [5,27,28,29].

Combining multiple markers was used in the current study to identify which and how many combinations of markers are most diagnostic. Different combinations used in the current study.

Using single marker, PCT was the marker with the highest sensitivity (93.7%). To increase the sensitivity of the test using 2 cytokines, the best results was achieved by using PCT with IL-10 in the second step, and the sensitivity increased to 98.7%. On using combination of three markers, the best combination (100% sensitivity and the highest specificity 90.1%), was reached by combining PCT, IL-10 and IL-13 in the third step. To this step we have got 100% sensitivity and the highest specificity. The addition of further markers to the test will decrease the specificity and will cost more. Therefore, the diagnostic utility of these three markers was excellent.

5. CONLUSION

The Levels of combination of PCT, IL-10 and IL-13 serum levels were with high predictive value for sepsis diagnosis. However, their evaluation in large scale study is warranted. The clinical impact of measuring these diagnostic markers for sepsis diagnosis must be carefully considered.

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