

Comparison Between Procalcitonin and Traditional Blood Biomarkers in Diagnosis of Sepsis in Iraqi Wounded Soldiers

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Abstract

Early diagnosis of sepsis is a very critical matter especially for soldiers in battle field; on the other hand, Procalcitonin is a new promising biomarker for fast and accurate diagnosis of sepsis. The aim of this study is to investigate the ability of Procalcitonin (PCT) to indicate sepsis earlier than blood culture and other traditional biomarkers and then get use of this in early diagnosis of sepsis. Procalcitonin concentration was measured in 44 sera of Iraqi wounded soldiers who were admitted to GHAZI AL-HARIRI Hospital for surgery, using ELISA kit of BioRay/USA, along with blood culture and other infection biomarker like C-reactive protein, ESR, W.B.C. count. Six patients had elevated PCT and 3 of them had positive blood culture, two of these three died. PCT has good correlation with other inflammatory biomarkers. It is our recommendation to accredit PCT as a very early biomarker of sepsis in Iraqi hospitals along with blood culture but earlier than it, and to use the quick test of PCT in battlefield to determine the priority of transmission of wounded soldiers to hospitals.

Key Words: Procalcitonin, Soldiers Patients, Sepsis, Blood Culture, Biomarkers, C-reactive protein.

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1. Introduction

A massive challenge is facing workers at critical care unit (CCU) and intensive care unit (ICU) in Iraqi hospitals that receive wounded soldiers of the Iraqi army in its late war against terrorism these days. This challenge is represented by the early diagnosis of sepsis and determining the very appropriate treatment of it, since sepsis has no specific signs or symptoms and since wounded soldiers had to stay at battlefield for long time after injuring and this makes them very susceptible to sepsis. According to the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3), sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection [1]. Blood culture requires (24- to 48-h assay time) and does not reflect the onset of organ dysfunction; furthermore, it may be negative in sepsis patients for many reasons, nevertheless culture is still important to identify the antimicrobial therapy. Automated culturing systems detect bacteria based on solution pH or the presence of CO₂, with detection times of 11–31 h and false-positive and –negative diagnoses in the 2–3% range [2, 3]. Here comes the importance of biomarkers as predictor and early markers of sepsis, although their results and role in sepsis diagnosis is controversial because of their lack of specificity [4].

Procalcitonin is a very promising new biomarker because of the rapid rising of its level in serum after bacterial insult with systemic consequences [5]. Despite the overall conclusion that it was premature to use biomarkers for sepsis diagnosis, the 2001 International Sepsis Definitions Conference added procalcitonin and C- reactive protein to the new diagnostic criteria of sepsis [6]. So it is suggested to be a much related biomarker in early diagnosis of sepsis especially in hospitals with emergency sections and intensive care units [7] [8]. Many studies have been conducted with procalcitonin and its clinical laboratory application [9, 10, 11, 12].

The main purpose of this study is to investigate the ability of PCT to indicate sepsis earlier than blood culture and then get use of this in early diagnosis of sepsis since rapid and accurate diagnosis is essential.

2. Materials and Methods

Patients: Forty-four Iraqi wounded soldiers were enrolled in this study; all of them were brought to Ghazi Al Hareery hospital for surgery in Baghdad with injuries ranging between the leg, arm, head and abdomen for the period between 1st of February – 25th of May 2015. Patients' ages were ranging between (18-45) years with a mean of (28.52±6.9). The exclusion criteria were: any soldiers with no sufficient demographic information and any soldiers with burns, while the inclusion criteria were: preliminary diagnosed by physician as suspected to have sepsis and consent of the soldiers to participate in the study. Clinically, the sepsis was diagnosed by the physicians at the hospitals as a preliminary step then it

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comes the role of laboratory investigations which was done by using blood culture + CRP. This is the criteria accredited in Ghazi Al Hareery Hospital for Surgery, the place where the study conducted. All the patients were admitted in the CCU (Critical Care Unit) or the ICU (Intensive Care Unit) and the difference between the two units is that ICU stands for Intensive Care Unit. A full questioner was taken from patients about age, residence, location of injury, site of injury and type of injury.

Sample collection: The study patients were admitted to hospital in Baghdad with 1-2 days after injuring, therefore, the blood withdrawal was done directly after the admission and after the first diagnosis of sepsis was done by physicians and certainly before amputation surgeries if there were any. Ten milliliters of blood were collected from each patient and distributed as following: 7 ml for blood culture, 1 ml for serology tests (CRP and PCT), and 2 ml were added to EDTA anti-coagulated tube for hematological test [ESR (Erythrocyte Sedimentation Rate) +PCV (Packed Cell Volume) +W.B. Cs (White blood Cells) count].

Laboratory Investigations:

Blood Culture

Seven milliliters for blood culture were injected directly into blood culture bottles (provided by AFCO /Jordan). Blood samples collected and inoculated in blood culture bottles, were incubated in the 37°C incubator. Aliquots were taken from positive bottles for Gram Stain and subcultured on solid media (Chocolate and MacConkey agar). Isolates grown from culture media were used for diagnosis.

Inflammatory Markers

CRP was performed using latex agglutination kit from INMESCO GmbH/Germany, as one of the most important infection biomarkers.

PCT Procalcitonin was estimated using (Human Procalcitonin ELISA kit) provided by RayBio/USA which is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human Procalcitonin in serum with normal range <500 pg/ml according to the manufacturer's specifications, and according to Meisner, 1999, PCT concentrations can indicate type and site of infection [13].

ESR, Hb, PCV, W.B.Cs count were performed directly at the same day of collection according to the routine laboratory procedures.

Statistical Analysis:

Analysis was performed by MINITAB version 13 for Microsoft windows. Frequency distributions were generated and presented in table form. Assessed parameters were described as follows: mean \pm SD or minimum, maximum and median.

3. Results and Discussion

Patients' ages were ranging between (18-45) years with a mean of (28.52 \pm 6) Table (1) represents the demographic distribution of wounds in wounded soldiers (the study group).

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Most of the soldiers were wounded in the north and western area of the country, 68.2% {43.2% Salahaddin +25% Anbar}. Extremities wounds represent the majority of whole wounds (68.2% {34.1% Upper limb +34.1% Lower limb}). The rests of wounds distributed between head and abdomen.

Out of 44 patients involved in this study, 3 patients only were reported as positive blood culture and that was distributed as (2 *Candida albicans* and 1 *Acinetobacter*) versus 6 patients were reported as elevated PCT concentrations ranging between 2000-5000pg/ml (normal value below 500 pg/ml). All the three patients with positive blood culture were having elevated PCT concentration. Two of the three patients with positive blood culture died. Figure (1) represents serum PCT concentrations in all patients which varied from 0 to 5000 pg/ml, with a mean of (525±1205) pg/ml. According to Meisner, 1999 classification of PCT concentrations and their relationship to type and site of infection (13), PCT concentration were distributed into 3 groups as shown in Figure (2). Mean ±SD, median, minimum and maximum of Hb, PCV, W.B.Cs and ESR values are shown in table (2).

Figure (3) shows the positivity of (CRP) where it was positive in 59.1% of patients and negative in 31.9% , the six patients with elevated PCT were within the 59.1% positive CRP.

Over the past 15 years, the use of PCT in identifying the bacterial or non-bacterial origin of systemic inflammation has been gaining widespread support, and it is likely this trend will continue. Through various studies, procalcitonin was one of the markers which have been suggested for more rapid diagnosis of sepsis [14], and as part of loyalty to our army, it is our duty as students and lecturers to participate with our knowledge in relieving our soldiers` pain, so it was this research.

Our data showed the elevation of PCT (≥ 2000 pg/ml) in 6 of the 44 patients, and according to Meisner classification this concentration or above indicates sepsis although only three of these six patients gave a positive blood culture (13). This certifies the role of PCT as a good early biomarker for sepsis knowing that PCT results can be yielded within the same day of sample collection while blood culture results yielded at least after 3 days. Two of the three patients with positive blood culture died. About the other 3 patients with high PCT concentration and negative blood culture , they might be at first stage of sepsis which called SIRS (Systemic Inflammatory Response Syndrome) in which no microbial culture is detected but the patients should reveal one or two of the following signs:

- Temperature $> 38^{\circ}\text{C}$ or $< 36^{\circ}\text{C}$
- Heart rate > 90 beats/min
- Respiratory rate > 20 breaths/min
- WBC count $> 12,000\text{c}/\text{mm}^3$, $< 4000\text{c}/\text{mm}^3$

By referring to the results, table (2) in particular, notice the elevation of W.B.Cs count and ESR rate in all six patients with high PCT concentration (negative and positive blood culture) rather than CRP which was positive in all six patients with high PCT concentration

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(negative and positive blood culture) in addition to the clinical signs which ensure at least the high temperature of the patients. This indicates the importance of PCT in early and accurate diagnosis of sepsis knowing that PCT concentration in serum reaches its peak after 6-12 hours after infection and that is much faster than blood culture as a diagnostic tool [15]. These results were in accordance with other studies like that of Chiesa *et al* (2015) who concluded that procalcitonin has a very important and promising role in early diagnosis of septicemia in newborns who are suspected to, by investigating the credibility of elevated concentrations of PCT in the blood of these neonates at intensive care unit [16]. Simon *et. al.* (2004) found that PCT level is more accurate with sensitivity of (88% vs. 75%) and specificity of (95% vs. 67%) than CRP levels by integrating several published works and by performing a meta-analysis study to compare the sensitivity and specificity of both procalcitonin (PCT) and C-reactive protein (CRP) levels as biomarkers in diagnosis of bacterial infection. (17). Raoofi *et al.* (2014) went even further by calculating positive and negative predictive values of procalcitonin serum level beside sensitivity and specificity, and that at first time and 72 h after admission to hospital and compare it with a blood culture test for diagnosis of sepsis. They recommended the adoption of procalcitonin serum level side by side with the patient's clinical symptoms as suitable criteria in sepsis diagnosis (18). In another prospective multicenter study performed by Sastre *et. al.* (2006) they revealed that PCT should not be used as the only marker of sepsis because its moderate diagnostic credibility and would be useful as part of a full sepsis evaluation (19). Back to our patients' category, Hope *et.al.*(2014) performed a prospective study involved 200 combat wounded soldiers who sustained extremity only wounds and revealed the role of PCT as a predictor for the development of Heterotopic Ossification (HO) in extremity wounds, and recommended the usage of PCT as a screening marker for the development of (HO) (20). Moreover, many studies evaluated the prognostic value of procalcitonin (PCT) level in trauma and multiple trauma patients and they found that long lasting elevated concentration of procalcitonin in the post-traumatic period, or its repeated increase, is a good marker of developing complications observed earlier than clinical manifestations (21,22). Our results are very encouraging to perform more studies on PCT and include it in sepsis diagnosis criteria in Iraq since it is not in the sepsis diagnosis criteria till now. We recommend using this biomarker, PCT, as an early diagnosis tool of sepsis in battle field to determine the priority of injured soldier's transmission to hospitals.

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Table (1): Demographic information of Patients.

characteristics	Percentages %									
	Baghdad	Wasit	DhiQar	Diayala	Najaf	Babil	Kirkuk	Anbar	Salahaddin	Karbala
Residence areas%	38.6	4.5	11.4	20.5	2.2	2.2	2.2	2.2	9	6.8
Location of Injury %	Baghdad		Salahaddin		Anbar		Diayala			
	9		43.2		25		22.7			
Site of Injury%	Lower extremity		Upper extremity		Head		Abdomen			
	34.1		34.1		4.5		4.5			
Amputation %	Yes					No				
	79.1					21.9				
Amputation Site %	Lower limb		Upper limb							
	34.1		4.5							

Table (2): The hematological parameters values.

Variable	Mean	SDV	Med.of the parameters	Min.of the parameters	Max.of parameters
Hb(g/dl)	11.371	2.614	10.833	6.000	17.000
PCV(%)	28.11	7.84	26.5	12.00	45.00
ESR (mm/h)	22.14	25.61	13	4.00	125.00
WBC(c/mm ³)	9.87	3.063	9.15	6.000	22.000

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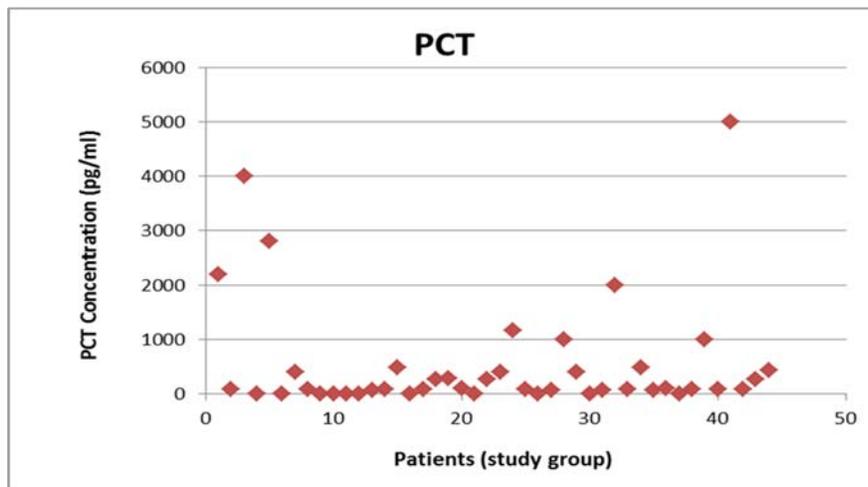


Figure (1): Serum PCT concentrations among patients with a mean of (525 ± 1205) pg/ml

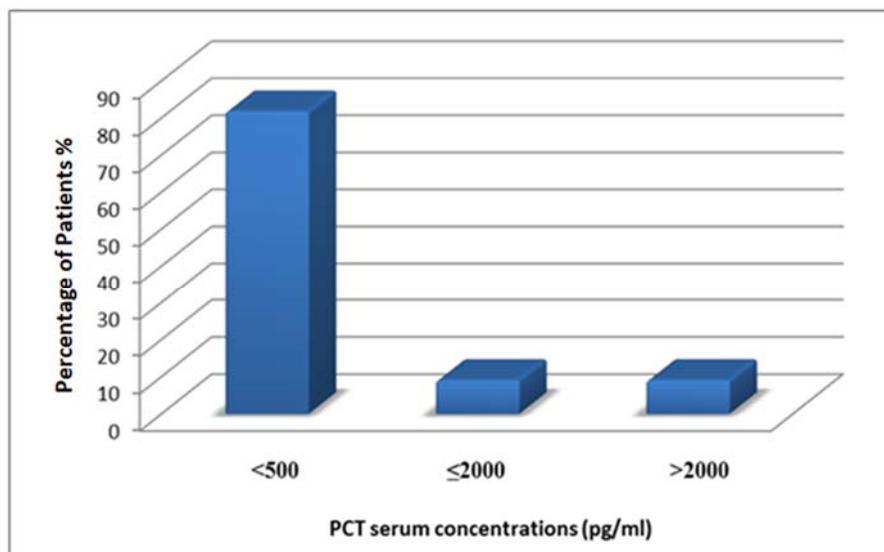


Figure (2): Histogram showed the Distribution of PCT concentration.

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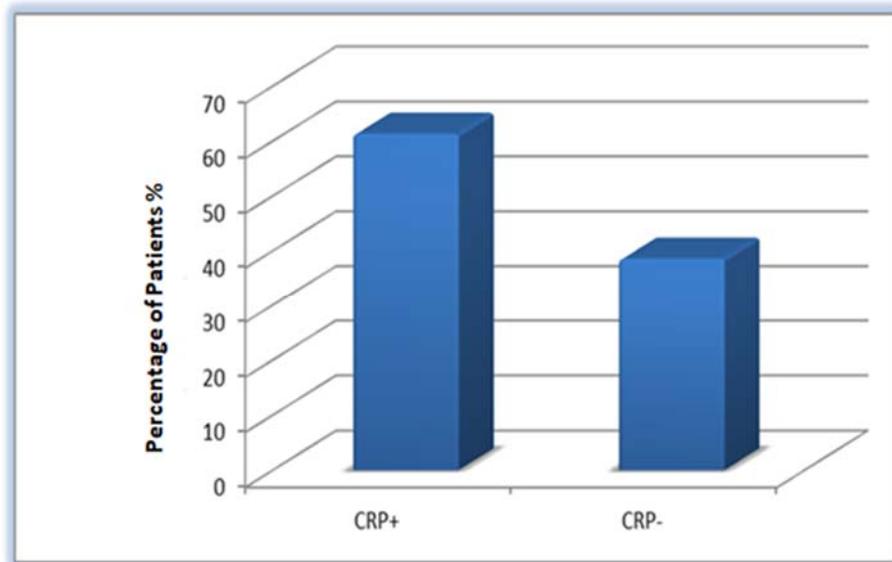


Figure (3): Histogram showed the percentage of CRP among patients

4. Conclusions:

It is our recommendation to accredit PCT as a very early biomarker of sepsis in Iraqi hospitals along with blood culture but earlier than it, and to use the quick test of PCT in detecting sepsis and serious bacterial infections.

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References

- [1] M. Singer,; C. Deutschman,; C. W Seymour,.; et al., The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 315 (8):801-810. 2016
- [2]ML. Wilson; S. Mirrett; LC. McDonald; MP. Weinstein; J. Fune; LB. Reller Controlled clinical comparison of bioMerieux VITAL and BACTEC NR-660 blood culture systems for detection of bacteremia and fungemia in adults. J Clin Microbiol 37 (6):1709–13. 1999

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- [3] A. Oberholzer; U. Steckholzer; M. Kurimoto; O. Trentz; W. Ertel Interleukin-18 plasma levels are increased in patients with sepsis compared to severely injured patients. *Shock* 16(6): 411–4. 2001
- [4] A. Leverssss and I. Mackenzie Sepsis: Definition, epidemiology, and diagnosis. *BMJ*. 335 (7625):879-83. 2007
- [5] M. Meisner Procalcitonin – Biochemistry and Clinical Diagnosis, 1st edition UNI-MED Science, Bremen, P.10-22. 2010
- [6] MM. Levy; MP. Fink; JC.Marshall; E. Abraham; D. Angus; D. Cook et al. SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Intensive Care Med.* (2003), 29(4):530-538. 2001
- [7] C. Pierrakos and JL. Vincent Sepsis biomarkers: a review. *Crit Care* 14 (1): R 15. (2010),
- [8] JC.Marshall and K.Reinhart Biomarkers of sepsis. *Crit Care Med* 37(7):2290-2298. 2009
- [9] FB.Rowther; CS.Rodrigues; MS.Deshmukh; FN. Kapadia; A.Hegde; AP.Mehta; VR. Joshi Prospective comparison of eubacterial PCR and measurement of procalcitonin levels with blood culture for diagnosing septicemia in intensive care unit patients. *J Clin Microbiol.* 47(9):2964–2969. 2009
- [10] A. Jacquot; JM. Labaune; TP. Baum; G. Putet; JC. Picaud Rapid quantitative procalcitonin measurement to diagnose nosocomial infections in newborn infants. *Arch Dis Child Fetal Neonatal Ed.* 94:F345–F348. 2009
- [11] AP.Georgopoulou; A. Savva; EJ. Giamarellos-Bourboulis; M. Georgitsi; M. Raftogiannis; N. Antonakos; et al. Early changes of procalcitonin may advice about prognosis and appropriateness of antimicrobial therapy in sepsis. *Crit Care* 26:331.e1-7. 2011,
- [12] C.Clec’h; F. Ferriere; P. Karoubi; JP. Fosse; M. Cupa; P. Hoang; Y. Cohen Diagnostic and prognostic value of procalcitonin in patients with septic shock. *Crit Care Med* 32(5):1166-1169. 2004
- [13] M. Meisner Procalcitonin: Experience with a new diagnostic tool for bacterial infection and systemic inflammation. *J Lab Med* 23 (5): 263-272. 1999
- [14] S.Canan,; G.Hulya,; S. Ercan,; Ugur and K. Bunyamin,. Usefulness of procalcitonin for diagnosis of sepsis in the intensive care unit. *Crit. Care.* 7(1): 85-90. 2003
- [15] SH. Hoeboer; Van der Geest PJ.; D. Nieboer; AB. Groeneveld The diagnostic accuracy of procalcitonin for bacteraemia: a systematic review and meta-analysis. *Clin Microbiol Infect.* 21(5):474-81. 2015
- [16] C. Chiesa; A. Panero; N. Rossi; M. Stegagno; M. De Giusti; J. Osborn; L. Pacifico Reliability of Procalcitonin Concentrations for the Diagnosis of Sepsis in Critically Ill Neonates. *Clinical Infectious Diseases* 26 (3):664–72. 1998
- [17] L. Simon; F. Gauvin,; DK. Amre; P. Saint-Louis; J. Lacroix Serum procalcitonin and C-reactive protein levels as markers of bacterial infection: A systematic review and meta-analysis. *Clin Infect Dis.* 39 (2):206–217. 2004

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- [18] R. Raoofi; Z Salmani.; F Moradi.; A. Sotoodeh; S. Sobhanian Procalcitonin As A Marker For Early Diagnosis Of Sepsis. *Am.J. of Inf. Dis.* 10 (1): 15-20. 2014
- [19] Sastre, J.L. Procalcitonin is not sufficiently reliable to be the sole marker of neonatal sepsis of nosocomial origin. *BMC Pediatrics*, 6(16): 1-7. 2006
- [19] D. Hope; E. Polfer; S. Safford; E. Elster; F. Lisboa; T. Brown; Forsberg J. Procalcitonin Levels in Serum Predict Heterotopic Ossification in Combat Related Extremity Wounds. *ORS Annual Meeting.* (2014), <http://www.ors.org/Transactions/60/1090>.
- [20] JV. Sakran; CP. Michetti; MJ. Sheridan; R. Richmond; T. Waked; T. Aldaghas; A. Rizzo; M Griffen.; SM. Fakhry The utility of procalcitonin in critically ill trauma patients. *J Trauma Acute Care Surg.* 73(2):413-8. 2012
- [21] M. Wojtaszek; G. Staśkiewicz; K. Torres; K. Jakubowski; O. Rącz; E. Cipora Changes of procalcitonin level in multiple trauma patients. *Anaesthesiol Intensive Ther.* 46 (2):78-82. 2014.

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