# APPLICATION OF PROCALCITONIN (PCT) - Q TEST FOR EARLY DETECTION OF BACTEREMIA AND SEPSIS

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**ABSTRACT**

*Procalcitonin (PCT) serum concentration increases abnormally in bacteremia, severe sepsis and septic shock and was described as an inflamation-induced protein.*
*The aim of this study is to evaluate the clinical application of PCT-Q test for determination procalcitonin (PCT) serum level in patients with suspected sepsis.*
*Fifty six patients according the criteria for sepsis (ACCP/SCCM,1992) and 19 controls were included. All patients undergo the bloodculture. Body temperature, leukocyte count, C-reactive protein (CRP) and procalcitonin were determined the same day. We used commercial solid-phase immunoassay B.R.A.H.M.S. PCT-Q (B.R.A.H.M.S.-Diagnostica GmbH, Henningsdorf,Germany) for semi-quantitative and rapid measurement of PCT.*
*Predominant causes of sepsis were infections of lower respiratory tract, surgical interventions, polytrauma. The data shows in 73,6% association with bacteremia and abnormally elevated PCT serum level. The results of extremely high PCT level was obtained for 28 (50%) of patients The 17,8% of patients , clinically suspected for sepsis, has high level of PCT, but negative bloodcultures. It was found 8 (14,2%) cases with low PCT level and positive blood cultures. The routine laboratory parameters and physical examination in many cases fail to establish the diagnosis of sepsis . PCT is more specific and sensitive marker of systemic bacterial infections then acute phase protein CRP.*

*The PCT-Q test is ease to use and its quick, validity results are important support for diagnosis of sepsis in early phase. In this way improved the diagnosis, monitoring and therapy of patients in risk.*

## Introduction

Sepsis is the main reason for the development of multiple organ failure and mortality in Intensive Care Unit (ICU). Adequate antibiotic therapy is the basic element of rational treatment (17). Sepsis can be difficult to distinguish from other noninfections conditions in critically ill patients. Infections and sepsis are accompanied by clinical and laboratory signs, such as changes in body temperature, leucocytosis,CRP, tachycardia but

***Abbreviations:*** ACCP/SCCM-American college of Chest Physicions/Society of Critical Care Medicine; PCT- procalcitonin, ICU-Intensive Care Unit, SIRS-Systemic inflamatory response syndrome, APACHE II score-Acute Physiology and Chronic Health Evaluation, SOFA score- Sepsis-related Organ Failure Assessment.

they often provide low discrimination in the diagnosis of infection (2,6,7).
In these cases early diagnosis and antibacterial therapy are very important. Patients who are doubtful to have these conditions, usually undergo blood cultures before the initiation of the therapy. Unfortunately, blood cultures,so specific for the detection of bacteremia, have a sensitivity of only 25-42% (28,29). Clinical signs of sepsis may develop without bacteriological evidence of infection and negative results do not exclude the presence of infection or sepsis (30). A rapid, early and reliable test for detection of bacteremia and sepsis is necessary.
PCT, a propeptide of calcitonin, has recently been proposed as a marker of severe sepsis, septic shock and non-viral infections when systemic manifestation are present ( 4,14,31).
The molecule, a 116-amino acid polypeptide with a molecular weight of approximately 13 kDa, consists of the fregments katakalcin, calcitonin and N-terminal residue. Active calcitonin (32 amino-acid peptide) is produced from PCT by means of specific proteolytic enzymes in the C-cells of the thyroid gland. During sepsis or severe bacterial inflamation, under the influence of endotoxin and cytokines the last proteolytic step is inhibited and the precursor peptide procalcitonin and its fragment katacalcin and N- procalcitonin are liberated into the circulation (21,25, 26).
The exact site of procalcitonin production during sepsis is as yet unknown. However ,the liver or neuroendocrine cells in the lungs are possible sites of extrathyroidal procalcitonin production in septic patients (4,5)
Synthesis of PCT is induced by bacterial endotoxins and exotoxins as well as by tumor-necrosis factor-alpha (11). The normal value in healthy humans is below 0,1 nag/ml(4). PCT, which is not detectable in the plasma of healthy individuals,is consistently released into the bloodstream 3-4 h after a single injection of endotoxin. Thus endotoxin induces the release of PCT systematically, and that the increase in PCT associated with septicemia in patients may be mediated through the effect of endotoxin (11). Values over 10,0 nag/ml were found with systemic infection or in septic patients.
The measurement of procalcitonin levels is simple and can be undertaken in routine laboratories. There are two commercial methods: immunoluminometric assay (LUMI test PCT, B.R.A.H.M.S. Diagnostica,Berlin) and PCT-Q B.R.A.H.M.S. test ( 20,22).

***The aim*** of the present study was to investigate the clinical application of PCT-Q test for determination of PCT level in patients with suspected sepsis.

## Materias and Methods

Fifty six adult patients ( 29 females and 27 males) according the criteria for sepsis adopted at the 1992 ACCP/SCCM Consensus Conference in the USA (3) were included in the study. All of them were admitted in the ICU and undergo blood cultures.We have also 19 controls- patients with sterile blood cultures and no clinical data for suspected sepsis. **Table 1** shows the clinical manifestation of SIRS, sepsis,and severe sepsis (3).
Blood was collected in aerobic and anaerobic culture bottles (Soybean-Casein Digest Broth,BD BACTEC,USA) incubated for 7 days in BACTEC 9050 blood culture system (BD,USA). Growing isolates were identified and their susceptibility to antimicrobial agents were investigated by Mini API System (Bio Merrieux,France)and conventional methods.Extended-spectrum beta lactamases
( ESBL) production was determined with ATB-BLSE test (Bio Merrieux,France)

TABLE 1

**Clinical manifestation of SIRS, sepsis and severe sepsis**

| |
|---|
| SIRS<br>• Any two or more of the following:<br>• Body temperature >38 C or < 36 C<br>• Heart rate > 90 bpm<br>• Hyperventilation (respiratory rate >20 or PaCo2 < 32 mm Hg)<br>• WBC > 12, 000/mm3 or < 4, 000/mm3 or immature neutrophils > 10% |
| Sepsis = infection + SIRS (the infection is the cause of SIRS) |
| Severe sepsis = sepsis + organ dysfunction (the sepsis is the cause of organ dysfunction |

Methicillin-resistant Staphylococcus was investigated by Oxacillin-screen agar (BD,USA).

An episode of bacteremia was defined by the first positive culture in a series or by a new positive blood culture obtained > 48 hours after the admission in ICU. A source of infection was defined as one of the following: abdominal sepsis, respiratory tract infection, urinary tract infection, Catheter-related infection and others.

Clinical and laboratory data: The following data were gathered for all patients: demographic data (age and gender), heart rate, blood preasure, body temperature at the time the blood culture were taken. The data about leukocyte counts, creatinine, C-reactive protein (CRP) were obtained from the patient records.

PCT-level was measured by B.R.A.H.M.S. PCT-Q test. This is semi-quantitative one-step solid phase immunosorbent assay. A polyclonal sheep anti-calcitonin antibody is immobilized on a solid phase and a monoclonal gold-conjugated mouse-anti-katacalcin antibody is used as a tracer in the soluble phase (23). In this study the serum was used. The serum of the sample solublizes the tracer antibody when applied to the provided test area and proceeds into the test area. The antibody-antigen complex becomes visible when it is bound by the immobilized anti-calcitonin antibody in the area of the test-strip, where it develops a red coloured line at concentrations above 0,5 nag/ml.

The density of the colour corresponds to the concentration of PCT in the sample and was allocated the following concentrations by comparison with the reference scale, provided as a printout on a separate sheet . The non-bound tracer permeates into the area of the control line, developing a dark red second line, indicating the positive functioning of the assay.Category of PCT- value and interpretation of test-results are showed on the **Table 2** (23) .

The microbiological samples were taken as follow:

- In patients with pneumonia- endotracheal aspirate (ETA).
- In patients with surgical intervention, phlegmona,peritonitis or trauma- from drenage.
- Urinary tract infections - urine.
- Catheter-associated infection-from CV-catheter.
- Meningitis-cerebrospinal fluid (CSF).

We used the APACHE II score for comprehensive evaluation of clinical status(18) and SOFA score to assess organ dysfunction (33) of patients with suspected sepsis.

TABLE 2

**Category of PCT- value and nterpretation of test results using B.R.A.H.M.S. PCT-Q assay**

| Category | PCT ng/ml | Interpretation |
|---|---|---|
| I | < 0,5 | Severe bacterial infection unlikely<br>Severe sepsis or septic shock unlikely<br>Locally confined infection cannot be ruled out |
| II | 0,5 - 2 | Local bacterial or systemic viral infection possible.<br>Non-infectious SIRS<br>Severe sepsis or septic shock unlikely |
| III | 2 – 10 | Systemic bacterial, fungal or plasmodial infection likely<br>Most often severe sepsis or septic shock<br>In some cases a non-specific increase is possible after<br>Major surgery,severe polytrauma or burn injury,or<br>During prolonged cardiogenic shock |
| IV | >10 | Sepsis, severe sepsis or septic shock most likely<br>Multiple organ failure |

The results were statistically processed by analysis of variances and t-test of Student-Fisher. The results are presented as mean value+ standard deviation.

## Results and Discussion

Characteristics of the patients are presented in the **Table 3.**

From positive blood cultures were isolated : *Klebsiella pneumoniae* (n=12), *Escherichia coli* (n=6), *Staphylococcus aureus* (n=6) Coagulase-negative *Staphylococcus* (CoNS) (n=5), *Psedomonas aeruginosa* (n=4), *Acinetobacter baumanii* (n=2), *Enterococcus faecalis* (n=1). Concerning resistance of isolates : Seven of *K.pneumoniae* isolates produced Extended-spectrum beta-lactamases (ESBL); four of *S. aureus* and 2 of *CoNS* are methicillin-resistant.Typical characteristic of these nosocomial isolates is their multiresistance.

The **Table 4** presented the obtained infectious focus.

On the **Table 5** showed the data about APACHE II ,SOFA- score and the clinical symptoms of sepsis.

The patients with suspected sepsis has a higher leucocyte count ($p<0.03$) and higher CRP ($p<0.0005$) and creatinine-serum levels ($p<0.005$). No statisticaly significant difference between these patients and controls for the mean heart rate ($p=0.11$), mean arterial pressure ($p=0.12$), and body temperature ($p=0.13$). A high body temperature , a high heart rate, or a low systolic pressure did not predict bacteremia or sepsis. It can see that CRP serum level,the leucocyte count, the presence of focus of infection, SIRS or clinical sepsis associated strongly with bacteremia but lesser degree than PCT. CRP is a very sensitive marker of inflamation, but cannot be used to differentiate bacterial from

TABLE 3

**Characteristic of patients**

| N of patients 56 | Males 27 | Females 29 |
|---|---|---|
| Age 23 - 67 | Mean 45.5 | Over 60 years 3 |

| Reasons for the suspected septic conditions | | |
|---|---|---|
| Reason | Patients (n | % |
| Nosocomial pneumonia | 12 | 21.43 |
| Surgica, interventions | 8 | 4,.9 |
| Polytrauma | 8 | 14,29 |
| Gunshots trauma | 6 | 10.71 |
| Urinary tract infections | 6 | 0.71 |
| Catheter-related infections | 6 | 10.71 |
| Peritonitis | 3 | 5.36 |
| Pancreatitis | 3 | 5.36 |
| Phlegmona diabetica | 2 | 3.58 |
| Haemodyalysis | 1 | 1.78 |
| Meningitis | 1 | 1.78 |

other inflamation, because it reacts very unspecifically showing significant elevations after surgery, multiple trauma, in patients with tumor, as well as in the case of viral infections (21.27). PCT is a more specific and sensitive marker of systemic bacterial infection than acute phase protein such as CRP. WBC, body temperature are simple and safe measurement for detection and for monitoring inflamation. They havn't diagnostic benefit in ICU because of them unspecifc (2)

The **Table 6** presented the results from bloodcultures and PCT level. The data shows association with bacteremia (blood culture positive) and PCT serum level higher than cut off (0.5-2 ng/ml) –categories 3 and 4. PCT-Q test is very quick , the results obtained after 30 minutes. Bloodculture give positive results after 24-48 hours and final identification needed 72 h. There are 10 patients clinically suspected for sepsis, high level PCT, but blood cultures don't grow microorganisms. In our study 17.8% of blood cultures of patients with strongly suspected sepsis are negative and in these cases PCT-level data are most important for early decision for antibiotic treatment. In these cases we fallow the suggestion for initiation an intensified search for an infectious focus and adequate anti-infective and support therapy (23).Our data of sensitivity of PCT serum level to predict bacteremia is 73,.%, respectively lower than Liaudat's data for 92%(19).

It was found 8(14.2%) cases with low PCT level, but positive blood culture. The explanation of this fact may be the delayed response in infections du to Gram-positive bacteria (19). Another reason for low PCT level in patients with positive blood-culture is the previous antibiotic therapy (14). It may be also differentiation between the the time of the blood taken and the peak of PCT level in serum. Our decision in these cases is to treat the patients as a bacteremic/septic and strongly monitored their condition.

In this study 21 patients shows PCT 2-10 nag/ml and 7 cases->10 nag/ml.Serum levels above 10 nag/ml is the highest category of the concentrations and indicate diagnosis of "severe sepsis" or "septic shock (see table 1). The High sensitivity and specificity

TABLE 4

**The data obtained of infectious focus**

| Focus of infection | Clinical sample | Suspected sepsis patients (n) | Controls (n) | Total |
|---|---|---|---|---|
| Lower respiratory tract | ETA | 12 | 4 | 16 |
| Surgical intervention (peritonitis,pancreatitis) | DR | 10 | 3 | 13 |
| Polytrauma, gunshot (soft tissue,bone) | DR | 10 | 5 | 15 |
| Urinary tract infection (UTI) | UR | 6 | 7 | 10 |
| Catheter-related infection | CVC | 6 | 0 | 6 |
| Diabetic phlegmona | DR | 2 | 0 | 2 |
| Central Nervous system | CSF | 1 | 0 | 1 |
| Total | | 47 | 19 | 66 |

ETA-endotracheal aspirate; DR- drenage; UR- urine; CVC- central venous Catheter; CSF- cerebrospinal fluid.

TABLE 5

**The data about APACHE II and SOFA scores and the clinical symptoms of sepsis**

| Signs | Suspected sepsis patients ( 56 ) | Controls (19) |
|---|---|---|
| Heart rate (b/min) | 106.2 + 9.78 | 96.00 + 12 |
| MAP (mm/Hg) | 56.00 + 5.49 | 70.56 + 8.58 |
| Temperature (C ) | 38.61 + 0.26 | 37.21 + 0.69 |
| WBC (x 10 9 cells/ml) | 16.45 + 2.56 | 10.76 + 2.83 |
| C-RP ( ng/ml) | 143 + 98.0 | 81.75 +79.00 |
| Creatinine (mkmol/l) | 149 + 123 | 91.00 + 46 |
| APACHE II | 19.16+ 5.11 | 9.39 + 7.03 |
| SOFA score | 6.00 + 2.86 | 2.61 + 2.06 |

* MAP- mean arterial pressure; WBC- white blood cells count.

TABLE 6

**The data from bloodcultures and PCT serum levels**

| P C T (nag/ml) | Suspected sepsis patients | | | Controls |
|---|---|---|---|---|
| Categories | Blood culture (+) | Blood culture (-) | Total (n/%) | |
| < 0,5 | 2 | 7 | 9 (16.8) | 19 |
| 0,5 – 2 | 6 | 3 | 9 (16.8) | - |
| 2 – 10 | 21 | 6 | 27 (48.2) | - |
| > 10 | 7 | 4 | 11 (19.64) | - |
| Total | 36 | 20 | 56 (100) | 19 |

of the marker PCT supported by great number investigations (12,15,16,19,31).

We have one patient with bacterial meningitis and PCT level 2 ng/ml. The diagnosis confirmed by Slidex Meningite Kit (Bio Merrieux,France). A. Viallon et al.,1999 presented lower value of 0.,2 ng/ml to differentiate between bacterial and viral meningitis in adult patients. This value resulted in many false positive results (43%) (32).

The routine laboratory parameters and physical examination in many cases fail to establish the diagnosis of sepsis,especially in critically ill patients. During the more severe conditions in our study there is very good association between bacteremia and high PCT-serum level. PCT-Q test is useful for early positive result. CRP and PCT are parameters with different profiles with according to the degree of the sepsis and systemic inflamation. PCT rises up to 20h earlier than CRP in plasms (9, 10, 13, 30). A number of studies suggested PCT as a marker of severe infections and sepsis (8, 9, 24, 30) and for the follow up of patients in which a bacterial focus is complicated by signs of severe sepsis and septic shock at the bedside (8, 9, 31). This is possible in the early phase and help to made regular decision for therapy.

Our expiriance with bedside use of PCT-Q test indicate that the PCT level seems to be an important early marker for bacteremia in suspected sepsis patients, as well as especially in blood-culture negative sepsis. The defining of PCT serum level in an early phase help to diagnose and differentiate patients with risk of severe sepsis. The process of its discussing in corelation with other laboratory or clinical markers improved the diagnosis, monitoring and therapy of patients at risk.

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