Full Length Research Paper

Procalcitonin (PCT), C reactive protein (CRP) and its correlation with severity in early sepsis

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Sepsis is a common cause of death, and represents a potentially life threatening disorder. Biochemical markers useful to initiate early antimicrobial treatment are being studied. Procalcitonin (PCT) and C reactive protein (CRP) have been proposed as markers for this purpose. To establish the level of PCT and CRP in early sepsis and its correlation with the APACHE II scale, levels of PCT and CRP were taken from 39 patients with sepsis criteria. They were correlated with the severity scale of APACHE II. Cultures were taken prior to antimicrobial usage to corroborate the bacterial origin in patients who were admitted to the Internal Medicine Service of the North Central Hospital of Petróleos Mexicanos. Descriptive statistics were performed (mean and standard deviation for numerical values and percentages) for nominal values. Pearson correlation and relative risk tests were performed to determine correlations. 39 patients in total; 20 with positive cultures and 19 with negative; patients with positive cultures showed PCT levels above 0.5 ng/ml; negatives below this number. The mean for CRP was 128 mg/ml. Correlation of mortality/APACHE II r = 0.707 p = 0.01; PCT/APACHE II r = 0.523 p = 0.001. For cultures: CRP/culture r = 0.575 p = 0.0001, PCT/culture r =0.448 p = 0.004. Relative risk (RR): PCT > than 2 ng/dl and cultures RR= 4. The relative risk PCT >2 mg/dl and death RR= 3.3. Cultures and CRP>128 RR= 2.4; death and CRP > 128 mg/dl RR= 2. PCT and CRP values are useful markers to determine early gravity of an infectious illness; PCT is useful to demonstrate early form of bacterial processes.

Key words: Procalcitonin, C reactive protein, interleukin-1 B, tumoral necrosis factor, interleukin 6, Interleukin 1.

INTRODUCTION

Bacteria or fungi can cause severe diseases in humans. Bacterial infection may proceed from superficial colonization to local invasive infection and result in further systemic manifestations like sepsis. Mortality rates rise with increasing severity of inflammation. The most severe case of infection, complicated by systemic inflammation is severe sepsis and septic shock. However, to start therapeutic efforts, an early reliable diagnosis is necessary and such therapy may be expensive or invasive (for example broad spectrum antibiotic treatment, surgical focus removal, intensive care treatment, supportive therapy like recombinant human activated protein C). In the most specialized Intensive Care Unit (ICU) the mortality rate associated with sepsis is approximately 30%, reaching 50% in complicated infectious processes (Angus et al., 2001).

Currently the management of sepsis do not have a diagnostic method to quickly identify the causative agent as well as susceptibility to antibiotics (Sierra 2007; Becker et al., 2004).

SEPSIS

Sepsis represents itself in approximately 750.000 cases annually in the United States (Angus et al., 2001). Sepsis

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was defined as the systemic inflammatory response syndrome (SIRS) along with a suspicious infectious source. SIRS is defined as two or more of the following alterations: Temperature > 38°C or < 36°C; heart rate of 90 per minute; 20 breaths per minute, PaCo2 > 32 mmHg; and leukocytes > 12,000 /mm^3, or < 4000 /mm^3, or 10% immature neutrophils (band forms) (Sierra 2007). There exist systemic response markers in early sepsis that have high sensibility and specificity, and that can contribute to an opportune diagnosis and allow the early start of an antimicrobial therapy. PCT and CRP have been proposed to this end.

PROCALCITONIN

PCT corresponds to a group of proteins related to the calcitonin gene (CGRP) I and II. That are catalogued as calcitonin precursors; it is a protein of 116 amino acids, with a molecular weight of 13 kDa. After the transcription of the gen CALC-1, the mRNA codifies a protein of 16kDa and 141 amino acids called procalcitonin, which begins the signaling sequence that when separated from the molecule in the rough endoplasmic reticulum gives origin to PCT (Balc et al., 2003). The transcription of the CALC-1 gen generates PCT and consequently mature calcitonin.

This process is normally confined to the C cells of the thyroid gland and in the absence of infectious processes the traduction of this gene is solely promoted at this site by the neuroendocryne system (Simon et al., 2004).

Once the infectious process has started, the liberation of lipopolysaccarides from the bacterial membrane is promoted, which in turn liberates pro-inflammatory cyto-kines, mainly interleukin-1 B (IL-B) and tumoral necrosis factor alpha (TNF-A); which allow the initial expression of the monocytes of the CALC-1 gen with the consequent transcription of ARNm-CT and production of PCT (Linscheid et al., 2004; Clec´h, et al., 2004).

Once the traduction has initiated, PCT levels are detected in monocytes at 4 h, with its highest at 6 h and diminishes its production at 18 h (Clec´h, et al., 2004).

During serious infections PCT is produced in tissues other than the thyroid. In patients with prior thyroidectomy and serious infections, elevated levels of this prohormone can be found (Remolina-Schlig, 2005).

PCT has a half-time life in serum of 25 to 30 h, in contrast to the short half-time life of calcitonin which is 10 min (Becker et al., 2004). PCT levels are less than 0.1 ng/ml in absence of serious infection, but in serious infection increase to 6- 53 ng/ml (Remolina-Schlig, 2005). There exists a direct relationship between the levels of PCT with the severity of infection, even with the follow up of seriously ill patients, those who survived the infection had lower levels that those who died (Remolina-Schlig, 2005). Werra found that PCT levels > 1.5 ng/ml were associated with sepsis, with a specificity of 72% and a sensitivity of 100%.

C- REACTIVE PROTEIN

The direct determination of proteins and/or pro-inflammatory cytokines in serum has been used to perform the prognosis and diagnosis of several critical illnesses; among these C reactive protein (CRP) is the most widely used and the most accessible. This protein belongs to the pentatraxin family; it has five identical subunits codified by only one gene which is located in chromosome 1. These units associate themselves to form a stable pentameric unit, with a molecular weight of approximately 118 KD. It forms part of the innate immu-nity and its synthesis is induced as a response to tissue damage (Amescua et al., 2007). CRP level raises rapidly in response to inflammatory stimulus and this levels disminished rapidly when the inflammatory stimulus has resolved. It does not present a gender difference and values are not affected by other conditions such as anemia, polycitemia or erythrocyte morphology (Clyne and Olshaker 1999). It is synthesized by hepatocytes and the vascular endothelium and its expression is regulated by cytokines, particularly interleukin 6 (IL-6), and to a lesser degree by interleukin 1 (IL-1) and TNF-A (Gabay and Kushner, 1999).

PCR works by activating the complement cascade

Membrance attack complex is recognized by C1q and activates the classical pathway of complement. Also, it provides sites for union of the H factor, regulating the amplification of the alternative pathway and the C5 convertases. CRP synthesis begins 6 h after the inflammatory stimulus started and reaches peak level at 24 to 72 h. The halftime life is approximately 19hrs, in plasma is stable, without regard to food intake or circadian cycle (Amescua et al., 2007). Once the stimulus of IL-6 has ended it returns to normal values in an average of 7 days.

The mean concentration of CRP in healthy patients is 0.8 mg/L, but after a stimulus, increase this value up to more than 10,000 times (Amescua et al., 2007). CRP levels < 10 mg/L represents slight inflammatory processes, levels from 10 to 100 mg/L means moderate inflammatory processes and severe inflammatory processes have > 100 mg/L like sepsis.

MATERIALS AND METHODS

Type and study design

Descriptive study, observational, prospective and longitudinal. No patient was excluded.

Study population

18 to 90 year old patients admitted at the North Central Hospital of Petróleos Mexicanos (PEMEX) on Mexico City, recruited from the Internal Medicine department and intensive care, urinary tract infection was the septic focus most common. From April 1st through

June 31st 2009. The study was approved by our institutional review board and ethic committee.

Selection criteria

INCLUSION CRITERIA

Patients older than 18 years of age, with sepsis criteria and informed consent.

EXCLUSION CRITERIA

Thyroid cancer, failure to obtain informed consent, age <18 years, autoimmune diseases such as systemic erythematous lupus, rheumatoid arthritis, Crohn's illness or inflammatory bowel disease. Estrogen or steroid use.

Methods and data recollection instructions

Patients who met the inclusion criteria, has blood samples taken to determine PCT and CRP, as well as for sputum cultures, urine cultures, blood cultures for bacteria and fungi. For statistical purposes, positive cultures were given a value of 1 and negative cultures were given a value of 2; mortality was valued with 2 for patients who died and 1 for those who survived. PCT value measurement was accomplished through inmunoenzymatic assay (VIDAS ® BRAHMS PCT BioMerieux, France Place of manufacture, No. DASA5639 Series). PCT was first determined as described in detail elsewhere (Clec´h et al., 2004). According to the manufacturer, this immunoluminometric assay has a detection limit of 0.1 µg/L. Interassay and intraassay variations at both low and high concentrations were < 8 and 7%, respectively, C reactive protein was determined through CRP Life equipment (Hitachi modular. Automaticanalizer Roche, Type: Modular-000-G5, Part No. 767-0255, voltage AC400/230 3/NIPE V50H2. Power 14 KVA. Series No 2095-10) with the ELISA method. Normal values were between < 10 and 10 and 100 mg/L was taken as undetermined.

All patients had the determination of levels of CPR and the PCT, as well as the APACHE II scale to determine the severity of the disease.

Statistical analysis

Data were expressed as means and confidence intervals at 95%. Correlations between variables were assessed using r Pearson analysis. P values of < 0.05 were considered significant. Its PCT levels correlated separately with age, APACHE II, PCR and mortality through the implementation of the Pearson r. Levels were correlated with age C-reactive protein, leukocytes, APACHE II, PCT and mortality through the implementation of the Pearson r. Morality was correlated with the levels of leukocytes and the severity score APACHE II disease by applying the correlation r obtained Pearson. To interpret the relative risk of mortality for PCT and CRP we used the SPSS V.13.

The principal variables taken to relate with the index of illness severity (APACHE II) and mortality vs PCT and CRP. Values were compared among themselves separately obtaining specific statistical weight. PCT and CRP levels were obtained at patient admission. Mean tendencies for age and gender were performed.

PCT levels were correlated separately for age, APACHE II, CRP, cultures and mortality through the application of Pearson's r Test. Levels of C reactive protein were correlated with age, APACHE II, PCT, cultures, and mortality through the application of Pearson's r. Test.

Mortality was correlated with the index of illness severity APACHE II through the application of Pearson's r Test. Frequency of the germ was analyzed with respect to positivity of culture.

Relative risk for PCT will be obtained for mortality and cultures. Relative risk will also be performed for CRP with mortality and cultures. All the statistical analysis will be made with SPSS V.10.

Financial resources

PCT and CRP measurements were taken with resources from Mexico City's Sanatorium Durango.

RESULTS

A total of 39 patients; 17 male and 22 female. No patient was excluded. Average age was 64 years with SD ± 18. Minimum age was 19 years and maximum 94 years, 95% Confidence interval range of 8.4 - 5.64 with p = 0.005. PCT mean was 8.4 (±18) ng/dl; CRP: median 128(± 81) ng/dl; APACHE II: mean classification 19.3 (± 6); mortality 1.2 (±0.4). Of 39 patients, 20 showed positive cultures, (uroculture and/or hemoculture and peritoneal fluid culture). In 19 patients the cultures was negative. (Figure 1) The average for the CRP levels in patients with positive culture was 173.5 ng/dl with a minimum value of 22.5 ng/dl and a maximum of 329 ng/dl. Two cultures were positive to Candida. In patients with negative cultures, the mean value for CRP was 80.9 ng/dl with a minimum of 3.3 ng/dl and a maximum of 193 ng/dl.

Patients with PCT levels > 10 mg/dl, 23% died. The remainder of patients had an adequate evolution with a tendency towards getting better. The average hospital days stay was 8.9 days.

The following correlations were obtained through Pearson's r: Mortality/ APACHE II r =0.707 p = 0.01; PCT/APACHE II r = 0.523 p=0.001; PCT/mortality r = 0.303 p = 0.61; PCT/age r = 0.47 p = 0.037; CRP/APACHE II r=0.188 p = 0.252. CRP/mortality r = 0.288 p=0.76. CRP/age r = -131 p = 0.583. The correlation of CRP/ culture was r = 575 p=0.000. PCT/culture r = 448 p = 0.004. The relative risk of PCT values above 2 ng/dl and the cultures was of 4. The relative risk for PCT values greater than 2 mg/dl and death was of 3.3. The relative risk of cultures and CRP levels above 128 (median) was of 2.4. The relative risk for death and CRP values greater than 128 was of 2. (Table 1)

DISCUSSION

The results show that PCT and CRP are useful markers to determine if the infectious process is bacterial or not, by finding a relation between positive and negative cultures, and the values of these tests. A cohort value for CRP of 128 mg/dl was taken to determine relative risk of positive and negative cultures because this was our median. The relative risk and the correlation was significant for CRP as marker of a bacterial infectious process
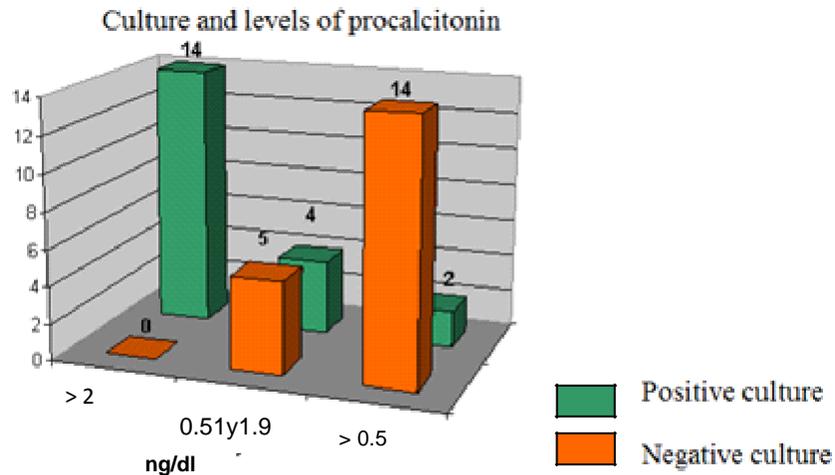
Figure 1. Cultures and procacitonin levels.
we see the relationship between positive cultures and levels of PCT. Noting that higher levels of PCT has the largest number of positive cultures.

Table 1. PCT levels for bacterial infection

Reference values	Probability of bacterial infection
Lower than 0.5 ng/ml	Not likely
0.5 – 2 ng/ml	Likely
Greater than 2 ng/dl	Highly likely

p= 0.000. CRP is a useful parameter to determine if a patient presents sepsis even though cultures are negative. A Portuguese study determined that values greater than 148 mg/dl of CRP are definitive to consider sepsis, and values below 24 mg/dl are negative for such process (Póvoa et al., 1998; Povoa, 2002). But the interval is very big and thus this value could be inespecific: more studies need to be performed with a special design to this purpose.

PCT levels greater than 0.5 mg/dl are useful to determine the type of infectious process (bacterial or not) (Brunkhorst et al., 2000). This association is close to 100% with levels greater than 2 mg/dl. In this study all the patients that presented positive cultures had PCT levels greater than 0.5 mg/dl and in most of them were greater than 2 mg/dl. The correlation of PCT with culture was significant (p= 0.004) and the relative risk was much greater than with CRP values. This is fundamental because by having this clear determination it is feasible to initiate early and efficient therapy prior to the infectious process being evident or having a positive culture.

These findings are related with those of another Mexican study (Remolina-Schlig, 2005) that reports that PCT presents a sensibility of 67 vs 71.8% of CRP and a specificity of 61 vs 66.6% for the discrimination of bacterial infection; the combination of both tests increments the specificity up to 82.2%; thus concluding that PCT

itself may not be a better marker for bacterial infection but in combination with CRP its utility is much better. Nonetheless this study is in accordance with international literature by showing that PCT is a useful marker in the diagnosis of a septic process by showing a sensibility of 78 % and a specificity of 94% comparing these values with CRP (Hatherill et al., 1999; Claeys et al., 2002; Bamonde et al., 2002). These studies have a more precise methodology towards the desired objectives and the sample number is much greater for which the statistical significance is much better; with this basis, the values found for CRP should be taken cautiously to discriminate between a bacterial infectious process from the non bacterial and we suggest taking cohort values greater than 128 mg/dl because this is already an illness severity parameter than coincides with that already reported in an Australian study (Ho et al., 2008). We tried to correlate PCT values with mortality, the result of such correlation through Pearson's r was .303 and p = 0.61 and thus was not significant; nonetheless when we correlated PCT levels with APACHE II SCORE the correlation was moderate and significant (p=0.001) and a relative risk of mortality of 3.3, which indicates that the higher the APACHE II index, the higher the PCT values.

In literature is reported that these values are correlated with severity of the illness as determined by APACHE II (Reinhard and Meisner, 2006) and in this manner it is

possible to predict a prognosis, they have even demonstrated that very high PCT values are related with septic shock and death considering a bad prognosis values greater than 10 ng/dl (Brunkhorst et al., 2002). In our study mortality was low and high PCT values were found (greater than 10 mg/dl) for which we consider than the appropriate therapy initiated precociously was related with an improvement and a better prognosis. These findings were not the objective of our study nonetheless more studies should be made to demonstrate than early therapy with PCT and CRP values are useful in reducing mortality. A study in Denmark demonstrated that patients with a septic process present less mortality risk with PCT levels less than 1 mg/dl, and increments in this value of more than 1 mg/dl per day in relation to the basal number are associated with an increment in mortality (Ulrik et al., 2006). The correlation of the PCT levels with those of CRP was not statistically significant. The relative risk of mortality with CRP was significative with values greater than 128 mg/dl. This value was the median for the determination of all patients. When these values are compared with current literature it is possible to affirm that patients with CRP levels greater than 128 mg/dl have a greater risk of death. In an Australian study a cohort value for the CRP values was obtained, mentioning a 7% risk of death with levels greater than 150 mg/dl and up to 21% when levels are greater than 300 mg/dl, with a mean in this study of 90 mg/dl and a mortality of 4.3% for this value (Seller-Pérez et al., 2005).

With regards to the utility of CRP as a prognosis marker, the majority of studies have not been able to demonstrate a correlation between CRP concentration at hospital enter and the survival (Pettilä et al., 2002).

Nonetheless, Vincent et al. (1996) confirmed the relationship between CRP concentrations, severity of multiple organic dysfunction and mortality. In our study these values showed levels greater than 128 mg/dl and having a relative risk for death of 2.

Conclusions

The diagnosis of sepsis prior to culture results and invasive procedures is feasible and could help in initiating an early therapy. This is fundamental in the high risk patients such as diabetics or inmunosupressed whose answer to infectious processes is much slower. PCT has shown to be a useful marker in differentiating septic processes of bacterial origin from those that are not, because the majority of patients with positive cultures has values greater than 2 ng/dl. CRP showed usefulness towards this end but being that this marker in nonspecific for infectious processes its usefulness is limited because any pathology with systemic inflammation of bacterial origin or not could increment its values. Nonetheless, published studies and ours coincide in that high CRP values (above 128) can indicate a bacterial infectious process and sepsis. PCT values were correlated with

APACHE II for which we concluded that the elevation of this marker is correlated with the severity of the illness and it is an early marker of sepsis. The determination of CRP although not correlated with APACHE II can be indicative of severity of illness by findings in our study that show a high relative risk. In this study a patient presented an initial PCT value greater than 43 ng/dl and survived, antibiotic therapy improved his prognosis in spite of this level, but posterior studies are necessary to demonstrate this finding. In our experience a marker for infection, evolution and prognosis has to be economically accessible and highly determinant with the purpose of initiating an effective and opportune therapy thus reducing the mortality and improving the prognosis of the patients with sepsis. The values of PCT associated with those of CRP could help us determine if the infectious process is of bacterial origin or not, if the patient has a septic process and to determine the severity of illness. The determination of biomarkers (CRP y PCT) could be a better tool to define sepsis activity and prognosis than the parameters currently used. Finally, we consider that it is necessary to perform studies with a bigger sample size because correlations are related to the sample size and possibly a greater number of patients could give us a better correlation with greater statistical weight.

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