Usefulness of Procalcitonin as a Diagnostic Marker of Pleural Effusion

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Abstract: Background Pleural effusions are common and are associated with many different diseases. Procalcitonin (PCT) is normally produced in the C-cells of the thyroid gland. It has recently become of interest as a possible marker of the systemic inflammatory response to infection. **Objective** Evaluating the usefulness and reliability of PCT level of pleural fluid & serum in determining the cause of pleural effusion**. Methods** This study was carried out on 54 adult patients with pleural effusion divided into five groups; transudate (n=6), empyema (n=9), Tuberculosis (T.B.) (n=8), parapneumonic effusions (PPE) (n=9) and malignant effusions (n=22). Levels of procalcitonin (PCT) were measured in serum & pleural fluid from the patients. **Results** Pleural fluid procalcitonin was highest in empyema, then in PPE, third in malignant effusions, fourth in tuberculous effusions & lowest in transudative effusions. Serum PCT showed similar trends. Pleural fluid & serum PCT levels positively correlated in patients with empyema & in patients with PPE. The optimal discrimination of patients with empyema and PPE could be performed at a cut-off point of pleural fluid PCT 0.9 and 0.08 ng/ml with area under the curve (AUC) of 0.93 and 0.66 respectively (sensitivity 80% and 78%, specificity 95% and 53% respectively) and at a cut-off point of serum PCT 0.08 and 0.054 ng/ml with AUC of 0.74 and 0.66 respectively (sensitivity 80% and 89%, specificity 60% and 33% respectively). **Conclusion** pleural fluid PCT is a good marker for early detection of infection in the pleural space and correlates with the serum PCT in patients with PPE or empyema. Pleural PCT has better diagnostic accuracy than the serum PCT in cases of PPE and empyema.
[Maha Yousif, Rana El-Helbawy, Amr Darwish, Heba Fathy and Nesreen El-Helbawy. **Usefulness of Procalcitonin as a Diagnostic Marker of Pleural Effusion.** *J Am Sci* 2012;8(7):382-387]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 58

Key words: Pleural effusion, parapneumonic effusion, empyema, procalcitonin

1. Introduction

Pleural effusions are common and can present as diagnostic challenges to the clinician. The development of disease-specific diagnostic biomarkers for pleural effusions is an active area of research. Among the potentially available laboratory parameters is procalcitonin (PCT).[(1)] It is considered to be an acute phase reactant protein, which structurally consists of 116 amino acids with a molecular weight of 13 kDa. [(2-6)] It is a propeptide of calcitonin produced by C-cells of the thyroid gland.[(7)] Normally, PCT levels are undetectable (<0.1 ng/mL) in healthy humans. In severe bacterial infections, intact PCT is found in blood.[(8,9)] One major advantage of PCT compared to other parameters is its early and highly specific increase in response to severe systemic bacterial infections and sepsis.[(10,11)] Thus, in sepsis increased PCT levels can be observed 3-6 hours after infectious challenge. As the septic infection resolves, PCT reliably returns to values below 0.5 ng/mL.[(12)] A correlation between serum PCT concentration and sepsis has been previously reported.[(13-14)] Nevertheless, few studies have investigated the diagnostic usefulness of PCT concentrations in body fluids. Therefore, this study aimed at evaluating the usefulness and reliability by PCT level of pleural fluid & serum in determining the cause of pleural effusion.

2. Patients and Methods

Fifty-four patients with pleural effusion were recruited from Chest and Internal Medicine Departments in Minoufiya University hospital, Egypt; during the period from July to December 2011. All had undergone diagnostic thoracentesis and venipuncture with measurement of PCT in pleural fluid and serum after obtaining ethical research approval from our hospital's ethics committee and informed consent from the patients.Nineteen patients needed pleural biopsy and 5 patients needed thoracoscopy for the diagnosis. Blood sampling was done at the same time that thoracentesis was performed. In patients submitted to more than one thoracentesis during the hospitalization period, only the results of the first tap were considered. The inclusion criteria were: adult patient, with one of the following diagnoses:

Transudative pleural effusion, malignant effusion, tuberculous effusion, parapneumonic effusion or empyema.

A transudative pleural effusion was defined as one with a ratio of lactate dehydrogenase

(LDH) in the pleural fluid to that in the plasma of less than 0.6 and a ratio of total protein in the pleural fluid to that in the plasma of less than 0.5; additionally, the LDH level needed to be less than two thirds of the plasma level, with no significant extrapulmonary infection. Malignant pleural effusion was defined as malignant cells detected on cytological

examination of the effusion or pleural biopsy and no evidence of obstructive pneumonia. Tuberculous pleuritis was defined as one or more of the following criteria: mycobacteria or caseating granuloma detected on pathological examination of pleural biopsy, positive mycobacterial culture from the pleural fluid or the pleural biopsy, sputum culture positive for mycobacteria, and both clinical and radiological response to anti-tuberculous treatment. Patients with distant infections were excluded. Para-pneumonic effusion (PPE) was diagnosed if the pleural effusion was accompanied by community-acquired pneumonia, but the effusion was not grossly purulent, no bacteria were detected on the Gram stain or culture of the pleural fluid. Empyema was defined as a grossly purulent pleural effusion accompanied by bacteria detected on Gram stain or a culture positive for bacteria. The exclusion criteria were neuro-endocrine tumors, non-infectious systemic inflammation as inhalational injury, pancreatitis, mesenteric infarction, burns, and recent surgery, patients with contraindication for thoracentesis, or had an inadequate amount of effusion drained for diagnostic procedures.

Collection of blood samples and pleural effusion fluids; Ten ml of whole blood and 10 ml of pleural fluid were withdrawn from all subjects into plain tubes and centrifuged at 3000 rpm for 5 min. Serum and pleural fluid clear supernatant were isolated and kept in Epindorfs at-20°C until analysis.

Measurement of PCT: Procalcitonin was measured in both serum & pleural fluid by ELISA (Human PCT ELISA kit. Glory science). The kit uses a double- antibody sandwich enzyme- linked immunosorbant assay (ELISA). The PCT was added to monoclonal antibody enzyme wells which were precoated with human PCT monoclonal antibody; incubated then PCT antibody labeled with biotin was added and combined with streptavidin- HRP to form immune complex. Incubation and washing were done to remove the uncombined enzyme. Chromogen solution A then B were added and changed the color into blue then finally yellow. The chromogen color and the concentration of human substance PCT of the sample were positively correlated.

Measurement of serum and pleural laboratory parameters: Serum & pleural fluid LDH levels were determined by kinetic colorimetric method (Biosystems, Spain) [15]. Serum & pleural fluid albumin levels measured by using brome cresol green colorimetric method (Diamond diagnostics, Germany)[16]. Serum & pleural fluid total protein levels were measured by colorimetric method (Diamond Diagnostics, Germany)[17]. Pleural fluid glucose levels were measured by GOD- POD Liquid colorimetric method (Diamond Diagnostics, Germany)[16]. Pleural fluid cholesterol levels were measured by colorimetric method (Spinreact, Spain)[18]. Pleural fluid bilirubin levels were measured by colorimetric method (Diamond Diagnostics, Germany) [19].

Statistical Analyses: Data were expressed as ean ± SD. The statistical difference between means was calculated using Chi squared test. The correlation between serum & pleural fluid PCT was done by Pearson correlation analysis. The Receiver-operating characteristic (ROC) curves were constructed to illustrate the predictive value of cut-off points of PCT. The point with the largest sum of sensitivity and specificity was chosen as a threshold. All these tests were used as tests of significance at $p<0.05$. [20] The data collected were analyzed by SPSS (statistical package for the social science software) statistical package version 11.

3. Results

The demographic data and the mean pleural fluid & serum levels of each group parameters are shown in table (I).

The pleural fluid LDH was highest in empyema (1026±776 U/L) and lowest in transudates (135±48 U/L) and the results were statistically significant (p-value < 0.05) between transudative effusion & PPE and between empyema & all other types. The total protein in pleural fluid was highest in PPE (4.7±1.9 gm/dL) and tuberculous effusion (5±1.1 gm/dL) and lowest in in transudates (2.5±0.7 gm/dL) and the results were statistically significant ($p<0.05$) between transudative effusions & PPE and between transudative effusions & tuberculous effusions.

Pleural fluid albumin was highest in PPE (4.3±1.8 gm/dL) and lowest in transudates (1.7±0.3 gm/dL) and the results were statistically significant ($p< 0.05$) between transudative effusions & all other types and between malignant& PPE. Regarding pleural fluid PCT (PF-PCT), it was highest in empyema (1.17±0.86 ng/ml), next in PPE (0.57±0.56 ng/ml), third in malignant effusions (0.09±0.14 ng/ml), fourth in tuberculous effusions (0.09±0.01 ng/ml) & lowest in transudative effusions (0.06±0.03 ng/ml) and the results were statistically significant ($p<$ 0.05) between empyema & T.B and between empyema & malignant effusion and between malignant effusion & T.B and between malignant effusion & PPE. Moreover, the serum PCT (S-PCT) was highest in empyema (1.4±1.73 ng/ml) next highest in PPE (1.06±1.2 ng/ml) and lowest in transudative effusions (0.09±0.0 ng/ml) and the results were statistically significant ($p< 0.05$) between malignant effusions & empyema and transudative effusions. & PPE.

Table (I): Comparison between Different Diagnoses Regarding the Studied Parameters

	Transudate	Empyema	T.B.	PPE	Malignant	*p*-value
Demographic data						
Patient n.	6	9	8	9	22	
Age (yrs)	44.5±14.4	58±17	57.7±2.5	53.6±16.6	53.3±15	≥ 0.05
Sex: M (n,%)	(1, 17 %)	(6, 67 %)	(4, 50 %)	(6, 67 %)	(10, 45.5%)	≥ 0.05
Pleural fluid biomarkers						
LDH U/L	135±48	1026±776	423±382	593±237	424±245	<0.05 bet. trans. & PPE empyema & all
Total protein gm/dl	2.5±0.7	3.8±0.6	5±1.1	4.7±1.9	3.7±1.6	<0.05 bet. trans & T.B Trans & PPE
Albumin gm/dl	1.7±0.3	3.8±1	4.2±0.7	4.3±1.8	3.2±1.2	<0.05 bet. trans & all Malig& PPE
Total bilirubin mg/dl	0.6±0.2	0.45±0.1	0.4±0.1	0.4±0.2	0.6±0.3	≥ 0.05
Cholesterol mg/dl	63±24	48±25	64±8	58±29	54±22	≥ 0.05
Glucose mg/dl	66±21	66.2±66	98±30	64±38	70±22	≥ 0.05
PCT ng/mL	0.06±0.03	1.17±0.86	0.09±0.01	0.57±0.56	0.09±0.14	<0.05 bet. empyema & TB, malig <0.05 bet. malig& TB, PPE
Serum biomarkers						
LDH U/L	625±210	748±394	292±142	417±262	669±374	≥ 0.05
Total protein gm/dl	8±1	7.5±1.2	7.9±0.9	8.7±2	8.2±1.3	≥ 0.05
Albumin gm/dl	5±1	4±1	4±0.6	4.3±0.9	4.4±0.8	≥ 0.05
PCT ng/mL	0.09±0.0	1.4±1.73	0.44±0.6	1.06±1.2	0.23±0.5	<0.05 bet Malig & empyema trans & PPE

Data are presented as mean ± SD. PPE: parapneumonic effusion, LDH: lactate dehydrogenase, PCT: procalcitonin, Trans.; transudate, Malig: malignant, T.B: tuberculosis

Table (II): Pearson Correlation Between Pleural and Serum Procalcitonin (PCT)

	Pleural PCT									
Serum PCT	**Transudate**		**Empyema**		**T.B**		**Parapneumonic**		**Malignant**	
	r	***p*-value**	**r**	***p* -value**	**r**	***p* -value**	**r**	***p* -value**	**r**	***p* -value**
	0.21	0.69	0.83	<0.05	-0.29	0.052	0.99	<0.001	0.35	0.11

When correlating pleural fluid & serum procalcitonin levels, a significant positive correlation was found in patients with empyema (r= 0.83, $p< 0.05$). Moreover, a highly significant positive correlation was found in patients with PPE (r= 0.99, $p< 0.001$). Table (II)

Table (III): Validity of PCT in Detecting Cases of Empyema and PPE

	Cut off value(ng/mL)	Sensitivity %	Specificity %	Accuracy %	PPV %	NPV %	AUC
Empyema							
• Serum PCT	0.08	80	60	62	20	96	0.74
• Pleural PCT	0.09	80	95	93	67	97	0.93
Parapneumonic effusion							
• Serum PCT	0.054	89	33	44	25	92	0.66
• Pleural PCT	0.065	78	53	58	29	90	0.66
Empyema +Parapneumonic effusion							
• Serum PCT	0.07	83	47	59	44	85	0.74
• Pleural PCT	0.075	83	58	67	50	88	0.84

PPV: positive predicted value, NPV: negative predicted value, AUC: area under the curve

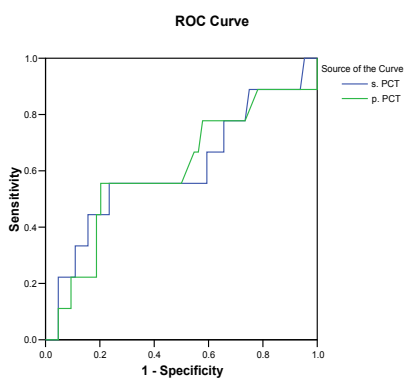

Fig: 1

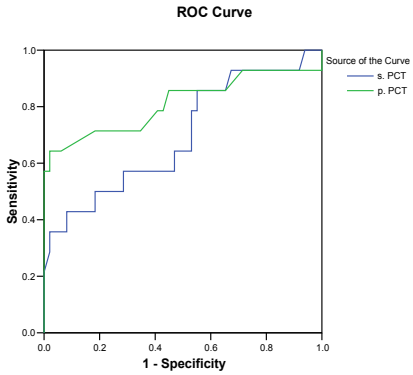

Fig: 2

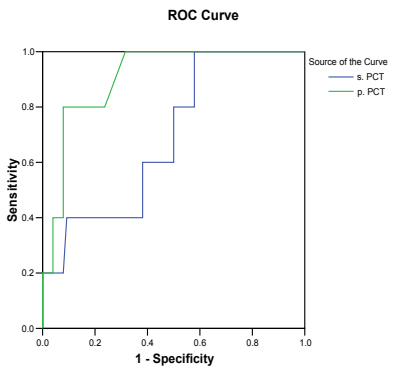

Fig: 3

Fig 1: Validity of procalcitonin in detecting cases of empyema from non-empyema cases
Fig 2: Validity of procalcitonin in detecting cases of PPE from non-PPE cases
Fig 3: Validity of procalcitonin in detecting cases of empyema and PPE from other case

The receiver-operating characteristics (ROC) curve analysis for an optimal discrimination of patients with empyema could be performed at a cut-off point of PF-PCT 0.09 ng/ml with area under the curve (AUC) of 0.93 (sensitivity 80%, specificity 95%) and at a cut-off point of S- PCT 0.08 ng/ml with area under the curve (AUC) of 0.74 (sensitivity 80%, specificity 60%). However, the ROC curve analysis for an optimal discrimination of (PPE) could be performed at a cut-off point of PF-PCT 0.065 ng/ml with area under the curve (AUC) of 0.66 (sensitivity 78%, specificity 53%) and at a cut –off point of S-PCT 0.054 ng/ml with area under the curve (AUC) of 0.66 (sensitivity 89%, specificity 33%). The optimal discrimination of patients with non-specific infection (empyema & PPE) could be performed at a cut-off point of PF-PCT 0.075 ng/ml AUC of 0.84 (sensitivity 83%, specificity 53%) and at a cut-off point of S-PCT 0.07 ng/ml AUC of 0.74 (sensitivity 83%, specificity 47%).(Table III, Figures 1-3)

4. Discussion

A number of biochemical markers of bacterial infection have been identified, though the validity of their measurement in pleural fluid remains unclear. Our study demonstrated that, PF-PCT was highest in empyema 1.17±0.86 ng/ml, next in PPE (0.57±0.56 ng/ml), third in malignant effusions (0.09±0.14 ng/ml), fourth in tuberculous effusions (0.09±0.01 ng/ml) & lowest in transudative effusions (0.06±0.03 ng/ml) and the results were statistically significant between empyema & T.B and between empyema & malignant effusion and between malignant effusion & T.B and between malignant effusion & PPE. Similar results were found between the S-PCT levels between the five groups with significant results between malignant effusions & empyema and transudative effusions. & PPE. Wang *et al.*[21] reported similar results in 76 patients with pleural effusion where PF-PCT concentrations were 5.147±3.056 ng/mL in empyema, 1.091±0.355 ng/mL in PPE, 0.130±0.069 ng/mL in tuberculous effusions, 0.241±0.071 ng/mL in malignant pleural effusions, and 0.188±0.077 ng/mL in transudative effusions. Topolcan*et al.*[22]found that the median PF-PCT levels were significantly higher in 26 patients with bacterial infection (0.67 ng/mL) than in 80 malignant effusions (0.14 ng/mL), 33 cardiac effusions (0.06 ng/mL) and 17 viral effusions (0.007 ng/mL). However, the authors did not fully describe the diagnostic criteria of the study population or calculate any discriminatory cut-off values for PCT.

Lin *et al.*[23]who studied S-PCT & PF-PCT levels in 82 patients retrieved from the emergency department found that their levels in the PPE group were significantly higher than those in the non-PPE group (PF-PCT, 0.37 vs 0.08 ng/mL, respectively [corrected p = 0.01]; S-PCT, 0.34 vs 0.1 ng/mL, respectively [corrected p = 0.0003]).

The significantly higher PF-PCT in empyema than PPE in the current study matches the results of Lin *et al.*[23] who demonstrated both PF-PCT and S-PCT levels increased with the severity of pneumonia classified according to the pneumonia severity index score in the PPE group.

However, in the subgroup analysis, there was no difference in the PF-PCT or S-PCT levels among patients with empyema, simple PPEs, and complicated PPEs. Moreover, the PF-PCT or S-PCT levels did not differ significantly among patients with tuberculous, malignant, and transudate Pleural effusions. Porcel*et al.*[24] investigated the PCT levels in different causes of pleural effusion and failed to

demonstrate any firm relationship between PF-PCT level and the cause of the pleural effusion. They explained their results by considering PCT as a potential biomarker of a state or a syndrome (e.g. severe sepsis) rather than an indicator of a disease.

Hence, we can consider PCT as suggestive indicator of empyema & PPE this is in concept of its early rise before culture results become positive. Early diagnosis of PPE & empyema enhances a favorable outcome and each of them has a different management line. Moreover, a high pleural PCT can differentiate between non-specific pleural infection & T.B.

When correlating PF-PCT & S-PCT levels in our study, a highly significant positive correlation was found in patients with PPE (r= 0.99, $p< 0.001$). Leon *et al.*[25] studied 47 ICU patients (12 pneumonia, 23 peritonitis, 12 non-infectious) and also found good correlation in S-PCT and PF-PCT levels (r=0.77, $p<0.0001$) .

Lin *et al.*[23] reported that S-PCT levels were highly correlated with PF-PCT levels (r=0.754, $p<0.0001$) among 45 PPE and 37 non-PPE patients. In Cakir*et al.*[26] study that excluded bacterial infections and focused on tuberculous pleurisy (18 out of 28 with T.B and 10 without T.B), the correlation between PCT levels in sera and pleural effusions was also significant (r=0.49, $p=0.008$).

In Wang *et al.* study [21], the correlation between S-PCT and PF-PCT levels was analyzed in 16 out of 76 subjects with both specimens available. Among these 16 patients, 12 had PPE and the other 4 had malignant pleural effusions. The analysis revealed a significant correlation between S-PCT and PF-PCT (r=0.967, $p<0.001$).

The present study supports the findings of others [21,23,25] for the significant relationship between serum and pleural effusion PCT levels the also suggests correlation between S-PCT and PF-PCT in PPE. These findings suggest that PF-PCT and S-PCT levels remain nearly the same in patients with different diseases, and that PCT is systemically expressed regardless of the presence of systemic sepsis or local inflammation, such as occurs in local malignant pleural effusions.[14]

The receiver-operating characteristics (ROC) curve analysis for an optimal discrimination of patients with empyema could be performed at a cut-off point of PF-PCT 0.09 ng/ml with area under the curve (AUC) of 0.93 (sensitivity 80%, specificity 95%) and at a cut-off point of S- PCT 0.08 ng/ml with AUC of 0.74 (sensitivity 80%, specificity 60%). However, the ROC curve analysis for an optimal discrimination of PPE could be performed at a cut-off point of PF-PCT 0.065 ng/ml with AUC of 0.66 (sensitivity 78%, specificity 53%) and at a cut-off point of S- PCT 0.054 ng/ml with AUC of 0.66 (sensitivity 89%, specificity 33%).

Lin *et al.*[23]showed that PF-PCT levels 0.18 ng/mL discriminated patients with PPEs from those with non-PPEs with a sensitivity of 67%, a specificity of 77% and an AUC of 0.752. Similar results were obtained by Wang *et al.*[21] where an optimal discrimination empyema and PPE from PPE could be performed at a cut-off point of 0.18 ng/mL with AUC of 0.776 (sensitivity: 69.7%, specificity: 72.1%). The lower S-PCT cut-off level in our study compared to other studies may be attributed to the localized infection within the pleural space in the PPE group. Interestingly, Lin *et al.*[23] showed that S-PCT had better diagnostic accuracy than PF-PCT and both correlated with the severity of pneumonia. In contrast to our finding that PF-PCT had better diagnostic accuracy than the S-PCT. This can be explained by differences in the severity of infection between patients in the two studies where in Lin *et al.* [23] the infections were more severe as patients were retrieved from the emergency department, moreover no data about previous antibiotic treatment in patients of our study that could decrease the S- PCT.

Study limitations

The present study should be interpreted in the context of it has the following limitations. Some PPE patients who received prior antibiotic therapy which seemed to decrease S-PCT rapidly because PCT has a half-life of 24 hrs. No data about the cause of effusion in the transudative group and a lack of data on duration of effusion. In conclusion, a high PF-PCT suggests the presence of empyema or PPE and correlates with the S-PCT in those patients. PF-PCT had better diagnostic accuracy than the S-PCT in PPE & empyema.

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6/11/2012