RESEARCH ARTICLE

Diagnostic significance of biochemical markers and pentraxin-3 in the differential diagnosis of malign, benign pleural effusion and empyema

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Abstract

Objective: To investigate the diagnostic significance of biochemical markers and pentraxin-3 in the differential diagnosis of pleural effusions.

Methods: The prospective clinical study was conducted at the Suleyman Demirel University, Isparta, Turkey, from January 2013 to June 2014, and comprised patients with pleural effusion. Pleural effusions were tested for glucose, protein, lactate dehydrogenase, and pentraxin-3 while simultaneous C-reactive protein and white blood cell levels were studied in the serums. Data was analysed using SPSS 22.

Results: Of the 96 patients, 48(50%) had malignant disease, 33(34%) had benign pleural effusion, and 15(16%) had empyema. In terms of glucose, protein, lactate dehydrogenase in the pleural effusions and C-reactive protein values in serums, significant differences were observed among the three groups ($p<0.05$). The pentraxin-3 levels in the empyema group was significantly higher than in the benign cases ($p<0.033$). No significant difference was observed in terms of the other variables between the groups ($p>0.05$).

Conclusions: Serum C-reactive protein and pentraxin-3 levels were not found to be individually conclusive in the differential diagnosis of pleural effusion. Also, lactate dehydrogenase levels were higher and glucose levels were lower in empyema.

Keywords: Pentraxin-3, Empyema, Pleural effusion, C-reactive protein, White blood cell.

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Introduction

Pleural effusion (PE) is caused by an imbalance between the secretion and absorption in the pleural cavity due to malignant or benign diseases. While congestive heart failure (CHF) and para-pneumonic effusions (PPE) are among the most common benign causes of effusions, pulmonary and mammary cancers are among the most common causes of malignant PE.[1,2] In 5-25% of PEs, the aetiology is unknown. Thus, new biochemical markers are required for an early diagnosis and treatment, and to prevent morbidity and mortality.[3] Pentraxin-3 (PTX-3) is a recently discovered acute phase marker resembling the C-reactive protein (CRP) in terms of structure and function. While CRP belongs to the family of short pentraxins, PTX-3 belongs to the long pentraxin family. Although CRP is a marker of inflammation, it is synthesised in the liver instead of being released from the area of inflammation. In contrast, PTX-3 is released from the macrophages, neutrophils, fibroblasts and vascular endothelial cells in the inflammation area. It is already known that the serum PTX-3 level is a new biochemical marker in inflammatory or vascular conditions such as heart failure, acute coronary syndrome, vasculitis and the systemic inflammatory response syndrome.[4-6] PEs are a common clinical finding among patients admitted to hospitals. PEs can cause many different complaints and disrupt the quality of life of the patients. Therefore, it is crucial to make the correct differential diagnosis and to plan the treatment without wasting time. There is still a need for new biochemical markers to facilitate differential diagnosis in PEs.

The current study was planned to investigate the diagnostic significance of biochemical markers and PTX-3 in the differential diagnosis of PEs.

Patients and Methods

The prospective study was conducted from January 2013 to June 2014 at the Department of Thoracic Surgery,

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Suleyman Demirel University (SDU), Isparta, Turkey, and comprised patients with PE.

After approval was obtained from the institutional ethics review committee, the sample size was calculated using G*Power 3.1 (Christian-Albrechts University, Kiel, Germany) with 95% power and 5% error level.[7,8] Age, gender, and diagnosis of the patients were noted, including malignancy status to decide about tube thoracostomy and/or surgery. All malignant PE patients were diagnosed histopathologically and were positive for PE cytology. Volunteer patients older than 20 years were included, and patients with congestive heart failure were excluded in the study. Tube thoracostomy was placed in order to diagnose and treat PE patients with exudative characteristic that fills more than 50% of the hemithorax, pushes mediastina and leads to recurrent respiratory problems. Drainage was stopped when it decreased to <100cc per day. PEs were tested for glucose, protein and lactate dehydrogenase (LDH), while simultaneous C-reactive protein (CRP) and white blood cell (WBC) levels were studied in the serums. Also, approximately 20cc of PE was sampled and sent to the microbiology lab. The PEs were centrifuged at 2000g in the microbiology lab, and after the removal of the formed elements, the supernatant serum phase was transferred into Eppendorf tubes and stored at -80^{O}C. All the PTX-3 levels in the PE serum were tested simultaneously in the microbiology laboratory of the Katip Celebi University, Izmir, Turkey, under double-blind conditions using the micro-enzyme-linked immunosorbent assay (ELISA) method in line with the quantitative standards based on the Human-PTX-3 ELISA Kit (Boster Biological Technology, CA) protocol and were measured at 450nm wavelength on the ELISA plate reader (Bio-Tek ELx808, USA). The contribution of PTX-3 to differential diagnosis of malignant, benign and empyema-dependent PEs was evaluated, but the association with survival was not evaluated in the Data was analysed using SPSS 22. Mann-Whitney U-test with Bonferroni correction at p=0.10 was used to find which groups caused the difference in the comparison of PTX-3 positivity and to compare the mean ages of the patients according to the gender. Statistical significance was set at p<0.05.

Results

Of the 96 patients, 48(50%) had malignant disease, 33(34%) had benign PE, and 15(16%) had empyema.

Table-1: Mean distribution and comparison of the biochemical data.

	n	Mean±SD	Median	Min.	Max.	*p*-value	Correlatin of Malignant PE, Benign PE, Empyema	*p*-value
Blood WBC								
Malignant PE	48	11.54±5.04	9.9	4	21	0.242	Malignant PE-Benign PE	
Benign PE	33	10.84±5.48	10.3	3	17		Malignant PE-Empyema	
Empyema	15	13.20±5.95	12.1	9	28		Benign PE-Empyema	
Blood CRP (ng/mL)								
Malignant	48	81.07±53.35	36.6	17	161	0.001	Malignant PE-Benign PE	0.002
Benign	33	62.25±63.02	68.3	3	130		Malignant PE-Empyema	0.003
Empyema	15	132.43±62.25	101.0	62	211		Benign PE-Empyema	0.001
Pleural fluid glucose (mg/dl)								
Malignant PE	48	109.23±75.95	118.5	40	389	0.001	Malignant PE-Benign PL	0.769
Benign PE	33	131.94±59.37	119.0	63	276		Malignant PE-Empyema	0.001
Empyema	15	18.73±16.21	12.0	0	50		Benign PE-Empyema	0.001
Pleural fluid LDH (U/L)								
Malignant PE	48	389.88±453.83	322.5	94	821	0.001	Malignant PE-Benign PE	0.284
Benign PE	33	279.30±299.15	267.0	65	646		Malignant PE-Empyema	0.001
Empyema	15	4917.73±11801.62	1774.0	727	33511		Benign PE-Empyema	0.001
Pleural fluid Protein (g/dl)								
Malignant PE	48	4.01±1.69	3.7	1	5	0.002	Malignant PE-Benign PE	0.374
Benign PE	33	3.14±1.49	3.1	1	4		Malignant PE-Empyema	0.002
Empyema	15	4.71±1.12	4.7	3	7		Benign PE-Empyema	0.001
Pleural fluid Pentraxin-3 (ng/mL)								
Malignant PE	48	21.38±27.95	17.76	3	37.88	0.046	Malignant PE-Beningn PE	0.072
Benign PE	33	14.03±19.55	6.71	1	23.96		Malignant PE-Empyema	0.182
Empyema	15	28.75±24.47	27.01	14	72.94		Benign PE-Empyema	0.032

PE:Pleural effusion; LDH: Lactate dehydrogenase; PTX-3: Pentraxin-3; CRP: C-reactive protein; WBC: White blood cells; SD: Standard Deviation.

Table-2: Diagnostic value of C-reactive protein (CRP) and pentraxin-3 (PTX-3) in the differential diagnosis of empyema.

	C-reactive protein (µg/mL)		Pentraxin-3 (ng/mL)	
Optimal cut-off points	**>39.9**	**95% CI**	**>48.9**	**95% CI**
Sensitivity	96.33	68.1 - 99.8	33.33	11.8 - 61.6
Specificity	41.03	30.0 - 52.7	98.06	87.8 - 98.6
+LR	1.58	1.2 - 2.1	6.75	3.3 - 13.8
-LR	0.16	0.02 - 1.1	0.7	0.3 - 1.9
+PV	23.3	13.4 - 36.0	55.6	21.2 - 86.3
-PV	97	83.9 - 99.9	88.5	79.9 - 94.3
AUC	0.665	0.525 - 0.805	0.642	0.481 - 0.804
AUC p	***0.043***	0.082		

AUC: Area under the curve

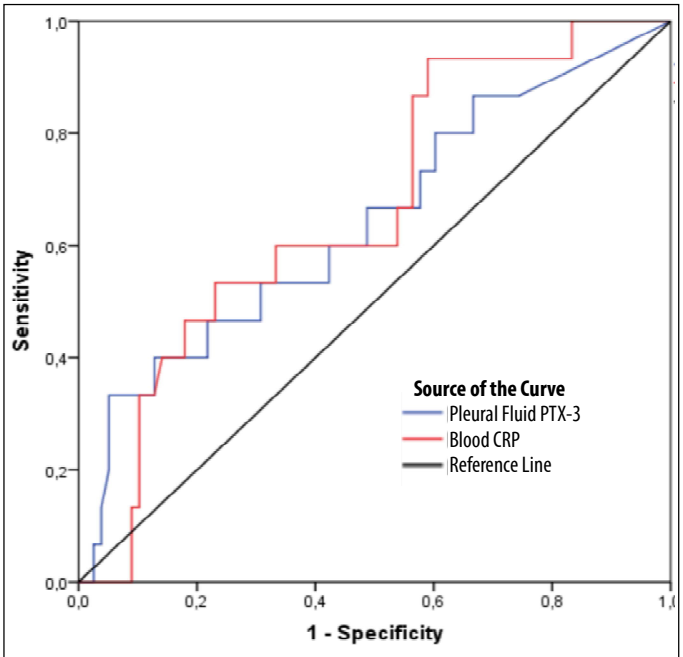

Figure: Receiver operating characteristic (ROC) analysis of C-reactive protein (CRP) and pentraxin-3 (PTX-3) in the presence of empyema.

Overall, 77(80.2%) subjects were males with a mean age of 62.341±6.06 (range: 20-88) and 19(19.8%) were females with a mean age of 61.63±14.78 (range: 31-81) ($p<0.05$). Among those with malignant PE, the condition was caused by pulmonary cancer in 18(37.5%), mesothelioma in 6(12.5%), mammary cancer in 12(25%), pancreatic cancer in 3(6.25%), and other malignant causes in 9(18.75%) patients.

In terms of glucose, protein, LDH in PE, and CRP values in serums, significant differences were observed among the three groups ($p<0.05$). No significant difference was observed among the groups regarding the WBC results. In the case of CRP values, all the paired comparisons between the groups resulted in statistically significant differences ($p<0.033$). In patients with empyema, PTX-3 was significantly higher than benign cases ($p<0.033$). The difference in the PTX-3 levels between benign and malignant PEs was statistically non-significant ($p>0.05$). Glucose values in PE were significantly lower in the empyema group compared to malignant or benign PE ($p<0.05$), while LDH values were significantly higher ($p<0.033$) (Table 1).

When the results from the diagnostic tests based on the CRP and PTX-3 values in the diagnosis of empyema were assessed, the sensitivity of CRP was 96.3%, specificity 41.03%, and the cut-off value was 39.9. The sensitivity of PTX-3 was 33.33%, specificity 98.06% and the cut-off value was 48.9. The area under the curve (AUC) of CRP was 0.665 (95% CI: 0.525-0.805; $p<0.05$). The AUC of the PTX-3 0.642 (95% CI: 0.481-0.804; $p>0.05$) (Table 2). The resulting receiver operating characteristic (ROC) curve were also charted (Figure).

Discussion

The present study demonstrated that serum CRP and PE PTX-3 levels were not individually conclusive in the differential diagnosis of PE. While PE is easy to detect radiologically and clinically, it is difficult to find out its aetiology. Some patients cannot be diagnosed even by using radiological methods and microbiological, biochemical and cytological analyses for PE. These diagnostic difficulties necessitate the investigation of new markers in PE. In literature, the diagnostic complications faced during the aetiological examination of PEs are mentioned and many cases are interpreted as idiopathic due to these complications.[6,9] Acute-phase proteins are various proteins produced mainly in the liver due to the increase in cytokines - mainly in interleukin-6 (IL-6) - resulting from an acute or chronic inflammatory incident. CRP starts to increase shortly after the onset of the inflammation to reach its maximum level within 48 hours. Its half-life (19 hours) does not differ between patients and healthy individuals and it rapidly responds to therapy with diminishing levels. These characteristics and its easy and cost-effective testing in many laboratories render CRP an important parameter in the diagnosis and treatment of inflammatory conditions.[10,11] In recent years, CRP has become more widely used to scan suspected inflammatory diseases, to evaluate the activity of inflammatory diseases, to diagnose and follow up infections, and in the differential diagnosis of

inflammations. Studies on CRP in PEs and the PE/serum levels are increasing in number. Thus, there are studies focussing on the serum CRP levels, PE CRP levels, and the PE/serum CRP separately or in combination in literature.[10,11,12]

On the other hand, PTX-3 is released from the macrophages, neutrophils, fibroblasts and vascular endothelial cells in the inflammation area. It is already known that serum PTX-3 level is a new biochemical marker in inflammatory or vascular conditions such as CHF, acute coronary syndrome, vasculitis and the systemic inflammatory response syndrome.[13] In a study[14] where serum CRP and PTX-3 levels were tested in 136 patients with ventilator-associated pneumonia who were mechanically ventilated for more than 48 hours, the threshold value of PTX-3 was >16.43ng/ml, specificity 74% and sensitivity 68.6%. It was reported that PTX-3 was not found to be superior to CRP in ventilator-associated pneumonia although it is an early marker in inflammation reaching its peak earlier than the CRP, and has a greater diagnostic value for mortality.[14] However in the present study, it was observed that the serum CRP and PE PTX-3 values were not strong markers in the differential diagnosis of PE.

Ozsu et al.[6] reported cut-off value of PTX-3 as 12ng/ml, sensitivity 88%, specificity 73%, and AUC 0.85 (95% CI: 0.769-0941) in patients with PPE. The study also reported that the PTX-3 value was correlated with the pleural neutrophil count and the serum CRP levels, and it was higher than the levels in malignant or other exudative PEs.[6] However, some studies have suggested that the high PTX-3 levels in certain malignant effusions are associated with the activation of the inflammatory pathways due to metastasis or proliferation.[11-13] In another study conducted on 118 patients with PEs, pleural PTX-3 value was more significant than CRP in the differential diagnosis of para-pneumonic and other malignant or tuberculosis-associated PEs. It reported PTX-3 cut-off value 25.00ng/mL, sensitivity 62%, specificity 81%, and AUC 0.74 (95% CI: 0.063-0.084).[15] A study on the diagnostic value and prognostic significance of pleural CRP in lung cancer patients with malignant PEs reported that pleural-CRP levels correlated with serum-CRP levels ($r=0.82$, $p<0.0001$).[12] For the differential diagnosis of malignant and benign PEs, the area under the ROC curve was greater for pleural-CRP than for serum-CRP. As a result, pleural-CRP was considered superior to serum-CRP in determining PE aetiology.[12]

The present study would have been more valuable if serum-CRP values had been compared by testing PE CRP values. This may be a limitation of the study. In another study conducted in order to distinguish exudative effusions from transudates in patients with PEs, the sensitivity of PE CRP was high, while its specificity was low.[16] In the present study, serum CRP cut-off value was >39.9 in patients with empyema, and the sensitivity was high (96,33%) in the serum while the specificity was low (41,03%). In another study specifying the viral agents using the multiplex-polymerase chain reaction (PRC) method on the nasal smears from 117 paediatric patients with lower respiratory tract infections, serum PTX-3 value in the patient group was correlated with procalcitonin and uncorrelated with CRP; and no significant difference was observed between the type of the viral infection and the PTX-3 levels.[17] The fact that PTX-3 increases independently from the viral agent may explain the low results observed in our study regardless of the bacterial, viral or fungal agents and the lower sensitivity in case of empyema.

Based on the results of the current study, further studies are recommended to describe the diagnostic value of PTX-3 in the differential diagnosis of malignant and benign PEs and empyema.

Conclusion

Serum CRP and PE PTX-3 levels were not individually conclusive in the differential diagnosis of PE. Also, LDH levels were higher and glucose levels were lower in empyema. However, since the specificity of PTX-3 and the sensitivity of CRP were higher in PEs due to empyema in comparison to benign and malignant effusions, their combination may be an alternative route in the diagnosis of empyema.

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