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Pleural Fluid Pentraxin-3 for the Differential Diagnosis of Pleural Effusions

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Background: Conventional biomarkers cannot always establish the cause of pleural effusions; thus, alternative tests permitting rapid and accurate diagnosis are required. The primary aim of this study is to assess the ability of pentraxin-3 (PTX3) in order to diagnose the cause of pleural effusion and compare its efficacy to that of other previously identified biomarkers.
Methods: We studied 118 patients with pleural effusion, classified as transudates and exudates including malignant, tuberculous, and parapneumonic effusions (MPE, TPE, and PPE). The levels of PTX3, C-reactive protein (CRP), procalcitonin (PCT) and lactate in the pleural fluid were assessed.
Results: The levels of pleural fluid PTX3 were significantly higher in patients with PPE than in those with MPE or TPE. PTX3 yielded the most favorable discriminating ability to predict PPE from MPE or TPE by providing the following: area under the curve, 0.74 (95% confidence interval, 0.63–0.84), sensitivity, 62.07%; and specificity, 81.08% with a cut-off point of 25.00 ng/mL.
Conclusion: Our data suggests that PTX3 may allow improved differentiation of PPE from MPE or TPE compared to the previously identified biomarkers CRP and PCT.

Keywords: PTX3 Protein; Pleural Effusion

Introduction

Pleural effusion is a common complication of many diseases, with an estimated 800,000 or more cases annually in the United States[1]. Parapneumonic (PPE), malignant (MPE), and tuberculous effusions (TPE) are the leading causes of exudative pleural effusion[2]. Although baseline studies including biochemical, microbiological, and cytological characteristics are helpful to differentiate the various causes of pleural effusion, the diagnostic confirmation is not always easily performed in clinical practice[3,4]. A variety of surrogate biomarkers have been proposed to facilitate differential diagnosis. Acute phase markers of inflammation, C-reactive protein (CRP) and procalcitonin (PCT) have recently been used to help differentiate infectious origin from other causes of pleural effusions[2,5-8].

Pentraxin-3 (PTX3) is one of the long members of the pentraxin superfamily. PTX3 was originally identified as an interleukin-1 (IL-1)/tumor necrosis factor (TNF)-inducible gene[9,10]. Some evidences suggest that PTX3 is involved in both innate immunity and inflammatory responses, serving as an acute phase protein. PTX3 is produced at the site of infection and inflammation by tissue cells, macrophages, monocytes, and dendritic cells in response to injury, trauma, and infection[10,11]. Recently, PTX3 was reported as a potential candidate biomarker to differentiate PPE from other causes of pleural effusions[12]. To our knowledge, PTX3 has not been compared with other biomarkers to differentiate the etiology of pleural effusions.

The primary aim of this study was to assess the clinical utility of PTX3 whether it can differentiate PPE from other causes of pleural effusions. Moreover, the diagnostic performance of PTX3 was compared with previously identified biomarkers.

Materials and Methods

1. Patients

A total of 120 pleural fluid samples were collected prospectively from 120 consecutive patients who underwent diagnostic thoracentesis at the Division of Pulmonology of Seoul St. Mary's Hospital (Seoul, Korea) between September 2008 and February 2011. Two of these patients were excluded from this study because the causes of pleural effusions were indeterminate or other causes were plausible.

All subjects provided written informed consent. The study protocol was approved by the Institutional Review Board at St. Mary's Hospital, the Catholic University of Korea (Project approval number: KCMC08BR162).

2. Diagnostic criteria of pleural effusions

The determination of the etiology of pleural effusions was based on widely accepted criteria[13]. Briefly, characterization of exudates was validated using the criteria set out by Light et al[14]. as follows: pleural fluid protein/serum protein ratio of >0.5; pleural fluid lactate dehydrogenase (LDH)/serum LDH ratio of >0.6; and pleural fluid LDH level more than two-thirds of the upper limit of the normal serum value.

Effusions occurring secondary to lung cancer (diagnosed by the presence of malignant cells in cytological examination or biopsy specimens, or by histologically proven primary lung malignancy with the exclusion of any other causes of pleural effusions) and other malignancies (effusions that were clearly secondary to other malignancies, with the exclusion of other causes) were considered as MPE. TPE was diagnosed by the presence of a positive stain, polymerase chain reaction or *Mycobacterium tuberculosis* culture in the pleural fluid, sputum or pleural biopsy. TPE was also identified by the presence of typical caseating granulomas in pleural biopsy, or if exudative lymphocytic effusion with high adenosine deaminase (ADA) levels (>40 IU/L) cleared in response to antituberculous therapy. PPE was identified by the presence of pulmonary infections associated with acute febrile illness, pulmonary infiltrates, purulent sputum and a response to antibiotic treatment and/or pleural fluid neutrophilia. Other causes of pleural effusions were excluded.

3. Measurement of biomarkers

Pleural fluids were obtained from the first successful thoracentesis prior to treatment, taken immediately after each patient's admission. Samples were subsequently analyzed for total and differential cell count, gram stain, pH, glucose, protein, LDH, ADA and lactate. Additionally, cytological examination and cultures for common aerobic and anaerobic bacteria, and the acid-fast stain, polymerase chain reaction for *M. tuberculosis*, *M. tuberculosis* culture were routinely performed on all pleural samples. Aliquots of pleural fluid were immediately centrifuged at 1,500 ×g for 15 minutes at 4°C and the supernatants stored at −80°C for analysis.

Pleural fluid cytokines including PTX3 (detection limit, 0.025 ng/mL), CRP (detection limit, 0.010 ng/mL), and PCT (detection limit, 27 pg/mL) were measured using commercial enzyme-linked immunosorbent assay kits (PTX3, CRP: R&D Systems, Minneapolis, MN, USA and PCT: BioVendor, Karasek, Czech Republic) according to the manufacturer's instructions after all pleural fluid samples were obtained. The technician who measured these biomarkers was blinded to the clinical diagnosis. Pleural fluid pH and lactate measurements were analyzed using a blood gas analyzer (ABL800 FLEX; Radiometer, Copenhagen, Denmark) immediately after pleural fluid drainage.

4. Statistical analyses

Demographic data in patients with pleural effusions are presented as mean±SE or number (%). To compare parameters between exudates of different origins, we performed a one-way ANOVA followed by a *post-hoc* Duncan's test. To estimate the ability to discriminate among pleural effusions, a receiver operating characteristic analysis was performed and the area under the curve (AUC) was calculated.

All biomarkers were subject to transformation when they did not fit a Gaussian distribution. Thus, after logarithmic transformation, biomarkers were applied to evaluate the significance of each group and back-transformed for data presentation. We considered a two-sided $p < 0.05$ to be statistically significant. All statistical analyses were performed using SAS version 9.2 (SAS Institute, Inc., Cary, NC, USA).

Results

1. Patient's demographics and baseline characteristics of pleural fluid

The study group included 79 males and 39 females with a mean age of 65.3 years. Of these, 15 (13%) had transudates, 38 (32%) had MPE, 36 (31%) had TPE, and 29 (25%) had PPE (Table 1).

Among 38 patients with MPE, 30 effusions occurred secondary to lung cancer (adenocarcinoma, 21; squamous cell carcinoma, 1; large cell carcinoma, 3; small cell carcinoma, 5), and 8 effusions occurred secondary to other malignancies (thyroid cancer, 3; breast cancer, 2; ovarian cancer, 1; endometrial cancer, 1; and stomach cancer, 1). Thirty-five out of 38 effusions were confirmed by cytological examination or biopsy specimens, and the others were clinically considered as MPE with the exclusion of other causes.

Patients with TPE were younger than those with other causes. Table 1 also lists the levels of pleural fluids pH, glucose, protein, LDH and ADA. Of the 36 patients with TPE, 23 cases were confirmed by the positive results of acid-fast stain, polymerase chain reaction for *M. tuberculosis*, or *M. tuberculosis* culture in the pleural fluid (n=12), or sputum or bronchoscopic studies (n=11). Other patients with TPE were diagnosed by exudative lymphocytic effusion with high ADA levels (>40 IU/L) that clinically cleared in response to anti-tuberculosis therapy.

Table 1. Demographics and baseline study of pleural effusion

	Transudates (n=15)	Class of exudates			p-value
		MPE (n=38)	TPE (n=36)	PPE (n=29)	
Demographic data (mean±SE)					
Age, yr	75±3	70±2	51±4	72±3	<.0001*†
Gender, n (%)					
Male	9 (60)	24 (63)	24 (67)	22 (76)	0.6556
Female	6 (40)	14 (37)	12 (33)	7 (24)	
Baseline study (mean±SE)					
pH	7.46±0.02	7.38±0.02	7.34±0.02	7.41±0.02	0.0031‡
Glucose, mg/dL	137±12	111±8	83±5	120±13	0.0197
Protein, g/dL	2.2±0.2	4.3±0.2	5.2±0.2	3.8±0.2	<.0001*‡
LDH, U/L	246±27	1272±126	1029±85	669±70	<.0001†‡
ADA, IU/L	9.4±0.7	21.7±1.6	92.8±5.9	24.2±2.6	<.0001*‡

p-value, difference between class of exudates; significant differences between *MPE vs. TPE, †MPE vs. PPE, and ‡TPE vs. PPE by multiple comparisons from ANOVA followed by a *post-hoc* Duncan's test.
MPE: malignant pleural effusion; TPE: tuberculous pleural effusion; PPE: parapneumonic pleural effusion; LDH: lactate dehydrogenase; ADA: adenosine deaminase.

Table 2. Biomarker levels of pleural effusions according to causes of pleural effusion

	Transudate (n=15)	Class of exudates			p-value
		MPE (n=38)	TPE (n=36)	PPE (n=29)	
PTX3, ng/mL	16.92±2.61	20.02±1.95	17.89±1.53	39.08±4.31	0.0004†‡
CRP, ng/mL	91.71±11.13	154.03±14.17	186.46±12.71	166.05±17.91	0.0437*‡
PCT, pg/mL	42.91±5.63	157.02±13.98	136.94±12.72	358.10±31.46	0.1537
Lactate, mmol/L	1.52±0.31	2.68±0.21	3.39±0.24	2.16±0.19	0.0024*‡

Values are presented as mean±SE.
p-value, difference between class of exudates; significant differences between *MPE vs. TPE, †MPE vs. PPE, and ‡TPE vs. PPE by multiple comparisons from ANOVA followed by a *post-hoc* Duncan's test.
MPE: malignant pleural effusion; TPE: tuberculous pleural effusion; PPE: parapneumonic pleural effusion; PTX3: pentraxin-3; CRP: C-reactive protein; PCT: procalcitonin.

2. Pleural fluid markers levels in exudates

The mean levels of pleural fluid PTX3 in patients with PPE were significantly higher than in those with MPE or TPE (39.08 ng/mL vs. 20.02 ng/mL and 17.89 ng/mL, respectively; p<0.001) (Table 2, Figure 1). Table 3 compared the operating characteristics of various biomarkers for differentiating PPE from other exudates. PTX3 yielded the most favorable discriminating ability (AUC, 0.74; 95% confidence interval, 0.63–0.84; sensitivity, 62.07%; and specificity, 81.08%) to predict PPE from MPE or TPE, with a cut-off point of 25.00 ng/mL.

Discussion

The purpose of this study was to assess the clinical utility of PTX3 for the differentiation of PPE from other causes of pleural effusions. Besides, the diagnostic performance of PTX3 was also evaluated with other surrogate biomarkers. We found that PTX3 has a more favorable diagnostic value for the differ-
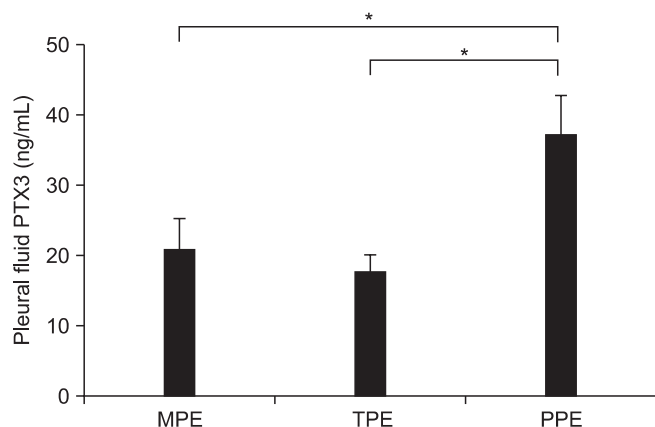

Figure 1. Levels of pleural fluid concentrations of PTX3 according to different etiologies of pleural effusions. MPE: malignant pleural effusion; TPE: tuberculous pleural effusion; PPE: parapneumonic pleural effusion; PTX3: pentraxin-3. *p<0.05.

entiation of PPE from MPE and TPE, compared to previously reported biomarkers including CRP, PCT, and lactate.

Pentraxins are a superfamily of conserved proteins that includes short and long members[10,15]. Classic short pentraxins include CRP and serum amyloid P component, and PTX3 was the first long pentraxin to be discovered[10]. Although both short and long pentraxins share a pentraxin signature sequence of high homology and function as acute phase proteins involved in innate immunity and inflammation, they are encoded by different genes, each with different cellular origins under different regulatory mechanisms[10].

The human *CRP* gene is located on chromosome 1q23, whilst *PTX3* is located on 3q24-28[10,15]. In contrast to CRP, which is primarily produced in the liver under the stimulatory influence of IL-6 and TNF-α, PTX3 is produced by a variety of cells and tissues, notably dendritic cells and macrophages, in response to Toll-like receptor engagement and inflammatory cytokines such as TNF-α and IL-1β[6,10,15-17]. Both short pentraxins and PTX3 can recognize microbial structures and activate complements, and can act as a humoral amplification loop of the innate immune response[15]. However, PTX3 production is more rapidly inducible than CRP, so this distinct characteristic can distinguish between these factors[9,15]. As CRP is primarily produced in the liver and may arrive in the pleural space from plasma diffusion, higher levels of CRP in pleural fluids may simply reflect higher systemic levels[6]. This may imply that CRP is unlikely to reflect regional inflammation, but rather the extent of systemic inflammation[6,15].

Several studies support PTX3 as a promising biomarker for acute inflammatory processes, as it is elevated during severe sepsis, septic shock, acute lung injury and acute respiratory distress syndrome[17,18]. The results of the present study were comparable with Ozsu's recent study, which reported that pleural PTX3 was positively correlated with pleural neutrophil percentage, and was increased much higher in PPE which was resulted from intense local inflammation than in MPE or in TPE[12]. They suggest PTX3 reflects local inflammation better than CRP. Furthermore, we demonstrated the diagnostic performance of PTX3 was superior to previous identified biomarkers, including CRP and PCT.

Pleural fluid CRP is a useful biomarker to the diagnosis and

Table 3. AUCs produced by receiver operating characteristics to predict PPE from MPE or TPE

	Optimal cut-off points	Sensitivity (%)	Specificity (%)	LR+ (95% CI)	LR– (95% CI)	AUC (95% CI)
PTX3, ng/mL	25.00	62.07 (44.41–79.73)	81.08 (72.16–90.00)	3.28 (1.89–5.69)	0.47 (0.29–0.75)	0.74 (0.63–0.84)
CRP, ng/mL	101.66	44.83 (26.73–62.93)	75.68 (65.90–85.45)	1.84 (1.04–3.26)	0.73 (0.51–1.04)	0.57 (0.43–0.70)
PCT, pg/mL	77.41	51.72 (33.54–69.91)	71.62 (61.35–81.89)	1.82 (1.10–3.02)	0.67 (0.45–1.01)	0.60 (0.48–0.72)
Lactate, mmol/L	2.50	77.78 (62.10–93.46)	54.17 (42.66–65.68)	1.70 (1.23–2.34)	0.41 (0.20–0.86)	0.67 (0.55–0.79)

AUC: area under curve; PPE: parapneumonic pleural effusion; MPE: malignant pleural effusion; TPE: tuberculous pleural effusion; LR+: likelihood ratio positive; LR–: likelihood ratio negative; PTX3: pentraxin-3; CRP: C-reactive protein; PCT: procalcitonin.

assessment of severity of PPE[2,5,19]. However, a number of studies investigating roles of CRP in pleural fluid were focused on the differentiation between complicated and uncomplicated PPEs[8,19,20]. In the present study, CRP levels in pleural fluid were also elevated in TPE and this finding is also noted in other previous reports[6,21]. It might be explained by profound tuberculous inflammation in TPE groups, represented by pH, glucose, protein and LDH. Levels of biomarkers, such as PTX3, CRP, PCT and lactate were higher in exudate than transudate, however, PCT levels were not significantly different among exudative pleural effusions. Therefore, novel biomarker adjuvant to conventional markers is clinically challenging, and pleural fluid PTX3 could be a promising biomarker for differentiating PPE from other exudates[12].

Several limitations of this study should be acknowledged. First, the number of cases was relatively small. Second, we did not measure biomarker levels in the serum samples; pleural fluid/serum ratios have been reported as useful in the differentiation of the causes of pleural effusion[6,7]. Third, the diagnostic criteria of pleural effusions were not firmly strict, meaning a bias may have affected the differential diagnosis. We permitted histological, bacteriological, and clinical diagnostic categories to enroll patients with MPE, TPE and PPE, depending on the physician's judgment.

In conclusion, we demonstrated that PTX3 could be a novel and promising biomarker that can differentiate PPE from other causes of exudative pleural effusions. Moreover, PTX3 showed more favorable differentiating ability compared to previously reported biomarkers such as CRP and PCT. Further large scale studies are needed to elucidate the role of PTX3 in exudative pleural effusion.

Acknowledgements

References

1. Marel M, Zrustova M, Stasny B, Light RW. The incidence of pleural effusion in a well-defined region: epidemiologic study in central Bohemia. Chest 1993;104:1486-9.
2. Daniil ZD, Zintzaras E, Kiropoulos T, Papaioannou AI, Koutsokera A, Kastanis A, et al. Discrimination of exudative pleural effusions based on multiple biological parameters. Eur Respir J 2007;30:957-64.
3. Light RW. Clinical practice: pleural effusion. N Engl J Med 2002;346:1971-7.
4. Hooper C, Lee YC, Maskell N; BTS Pleural Guideline Group. Investigation of a unilateral pleural effusion in adults: British Thoracic Society Pleural Disease Guideline 2010. Thorax 2010;65 Suppl 2:ii4-17.
5. Yilmaz Turay U, Yildirim Z, Turkoz Y, Biber C, Erdogan Y, Keyf AI, et al. Use of pleural fluid C-reactive protein in diagnosis of pleural effusions. Respir Med 2000;94:432-5.
6. Kiropoulos TS, Kostikas K, Oikonomidi S, Tsilioni I, Nikoulis D, Germenis A, et al. Acute phase markers for the differentiation of infectious and malignant pleural effusions. Respir Med 2007;101:910-8.
7. Lin MC, Chen YC, Wu JT, Ko YC, Wang CC. Diagnostic and prognostic values of pleural fluid procalcitonin in parapneumonic pleural effusions. Chest 2009;136:205-11.
8. Porcel JM, Vives M, Cao G, Bielsa S, Ruiz-Gonzalez A, Martinez-Iribarren A, et al. Biomarkers of infection for the differential diagnosis of pleural effusions. Eur Respir J 2009;34:1383-9.
9. Peri G, Introna M, Corradi D, Iacuitti G, Signorini S, Avanzini F, et al. PTX3, a prototypical long pentraxin, is an early indicator of acute myocardial infarction in humans. Circulation 2000;102:636-41.
10. He X, Han B, Liu M. Long pentraxin 3 in pulmonary infection and acute lung injury. Am J Physiol Lung Cell Mol Physiol 2007;292:L1039-49.
11. Garlanda C, Hirsch E, Bozza S, Salustri A, De Acetis M, Nota R, et al. Non-redundant role of the long pentraxin PTX3 in antifungal innate immune response. Nature 2002;420:182-6.
12. Ozsu S, Abul Y, Mentese A, Bektas H, Uzun A, Ozlu T, et al. Pentraxin-3: a novel biomarker for discriminating parapneumonic from other exudative effusions. Respirology 2013;18:657-62.
13. Papageorgiou E, Kostikas K, Kiropoulos T, Karetsi E, Mpatavanis G, Gourgoulianis KI. Increased oxidative stress in exudative pleural effusions: a new marker for the differentiation between exudates and transudates? Chest 2005;128:3291-7.
14. Light RW, Macgregor MI, Luchsinger PC, Ball WC Jr. Pleural effusions: the diagnostic separation of transudates and exudates. Ann Intern Med 1972;77:507-13.
15. Garlanda C, Bottazzi B, Bastone A, Mantovani A. Pentraxins at the crossroads between innate immunity, inflammation, matrix deposition, and female fertility. Annu Rev Immunol 2005;23:337-66.
16. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med 1999;340:448-54.
17. Mauri T, Coppadoro A, Bellani G, Bombino M, Patroniti N, Peri G, et al. Pentraxin 3 in acute respiratory distress syndrome: an early marker of severity. Crit Care Med 2008;36:2302-8.
18. He X, Han B, Bai X, Zhang Y, Cypel M, Mura M, et al. PTX3 as a potential biomarker of acute lung injury: supporting evidence from animal experimentation. Intensive Care Med 2010;36:356-64.

19. Porcel JM, Bielsa S, Esquerda A, Ruiz-Gonzalez A, Falguera M. Pleural fluid C-reactive protein contributes to the diagnosis and assessment of severity of parapneumonic effusions. Eur J Intern Med 2012;23:447-50.

20. Chen SC, Chen W, Hsu WH, Yu YH, Shih CM. Role of pleural fluid C-reactive protein concentration in discriminating uncomplicated parapneumonic pleural effusions from complicated parapneumonic effusion and empyema. Lung 2006;184:141-5.

21. Chierakul N, Kanitsap A, Chaiprasert A, Viriyataveekul R. A simple C-reactive protein measurement for the differentiation between tuberculous and malignant pleural effusion. Respirology 2004;9:66-9.