Diagnostic value of calprotectin in differentiation between benign and malignant pleural effusion

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Background Pleural effusion can arise as a result of more than 50 recognized causes and the differentiation between benign and malignant origin of the fluid is still a diagnostic challenge. The ability of tumor markers and other biological markers to make better diagnosis of malignant pleural effusion (MPE) remains questionable. Out of these, the calcium-related proteins S100-A8 and S100-A9 (the noncovalent heterodimer calprotectin) were demonstrated in a small amount in malignant not in benign pleural effusion.

Objectives This research aimed to assess the diagnostic value of calprotectin in the differentiation between infectious or benign and MPE.

Patients and methods Sixty patients were divided into group I: malignant and group II: infectious pleural effusions (which were further divided into group IIA: parapneumonic effusion and group IIB: tuberculous effusion) Quantitative measurement of calprotectin was done using the enzyme-linked immunosorbent assay technique in pleural effusion.

Results Pleural calprotectin level in MPEs (229.2±168.6 ng/ml) was significantly lower than its level of infectious pleural effusions (3202.2±1304.8 ng/ml; $P<0.001$). The cutoff value of calprotectin level for the diagnosis of MPE was less than or equal to 730.5 ng/ml, with 95% confidence interval and the area under the curve was 0.999, the corresponding sensitivity was 96.7 and the specificity was 100% ($P<0.001$).

Conclusion Calprotectin is a valuable biomarker in differentiating malignant from infectious pleural effusion.
Egypt J Bronchol 2019 13:382–387

Egyptian Journal of Bronchology 2019 13:382–387

Keywords: calprotectin, malignant pleural effusion, nonmalignant pleural effusion, pleural effusion

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Received 4 October 2018 **Accepted** 3 February 2019

Introduction

Diagnosis of pleural effusion remains a challenge. The list of the diseases they cause is big as well as it is heterogeneous. The more important difficulty in the diagnosis of exudative effusions is to differentiate benign from malignant effusion [1]. Most of malignant pleural effusions (MPEs) (90–97%) are exudative; they result from increased filtration from pleural vessels [2]. The initial semi-invasive method to diagnose exudative pleural effusion (thoracocentesis) allows cytological, microbiological, and biochemical analyses of the fluid [3]. More invasive procedures are required to diagnose exudative effusion with negative cytology particularly if malignancy is suspected. Closed pleural biopsy has a little diagnostic value and because of high diagnostic yield of thoracoscopy (≥90%), it is the method of choice [4].

Many studies have assessed the capability of tumor markers and other biological markers to make a better diagnosis of MPE [5]. Several proteins were demonstrated in malignant versus benign pleural effusion (BPE). Out of these, the calcium-related proteins S100-A8 and S100-A9 (noncovalent heterodimer calprotectin) were demonstrated in a small amount in MPE [6].

Calprotectin is a calcium- and zinc-binding protein of the S100 group heterodimeric complex [7]. Magne Fagerhol and colleagues first described calprotectin in 1980 [8], it is a 36 kDa protein with two 14 kDa and one 8 kDa chain of amino acid, these proteins were named so because they were 100% soluble in ammonium sulfate solution [9]. Calprotectin is heat resistant and resistant to proteolysis in the presence of calcium [10]. When neutrophil activation or endothelial adhesion of monocytes occurred, calprotectin begins to be secreted by a microtubule-mediated alternative pathway, thus acting as a marker for the influx of phagocytes into the site of inflammation [11], leading to increase its concentration in the plasma, serum, spinal fluid, synovial fluid, pleural fluid, urine, saliva, and stool during bacterial infection or inflammation in the relevant organs [12]. It has bacteriostatic and fungistatic properties that arise from its ability to sequester manganese and zinc [13]. It causes inhibition of metalloproteinases and chelation with zinc and manganese ions to inhibit microbial proliferation, as these metals are of vital importance for bacterial growth [14]. Calprotectin induces the

apoptosis both in malignant and nonmalignant cell lines [15].

The function of calprotectin in cancer biology is conflicting to some extent. It is a strong apoptotic factor when produced by immune cells. On the contrary, expression in cancer cells is related to tumor development, cancer invasion, and metastasis [9]. Its relationship with inflammation is obvious with an established proinflammatory role in various inflammatory states [16]. A high calprotectin level could be expected in a patient with inflammatory disorder [15]. It could be reasonably expected to find high quantities of this protein in MPE because it is also concerned with inflammation-associated carcinogenesis [9].

The higher levels of calprotectin found in BPE could attribute to the antimicrobial role of this protein. When neutrophil dies as a policy to suppress the growth of various fungal and bacterial pathogens, a massive amount of calprotectin is released [17]. Accordingly, calprotectin levels were high in parapneumonic and tuberculous pleural effusion and low in additional benign noninfectious etiologies [18].

Aim of the work

The objective of this study is to clarify the diagnostic value of calprotectin in the differentiation between infectious or benign and MPE.

Patients and methods

This study was conducted on 60 patients with pleural effusion admitted at the Chest Department, Benha University Hospital during the period from December 2016 till January 2018. Ethical research approval from Benha University Hospitals ethics committee and informed consent from the patient were obtained.

The patients were classified according to their final diagnosis into two groups.

(1) Group I: 30 patients with MPE.
(2) Group II: patients with infectious pleural effusion who were subdivided into two groups:
    (a) Group IIA, 15 patients with parapneumonic pleural effusion.
    (b) Group IIB, 15 patients with tuberculous pleural effusion.

Patient with any of the following criteria was excluded [19]:

(1) Under treatment with anticancer chemotherapy.
(2) Under treatment with antituberculous drugs.
(3) Using glucocorticoid and other anti-inflammatory drugs.

All patients were subjected to the following:

(1) Thorough medical history: age, sex, residence, occupation, smoking, and other particular habits of medical importance.
(2) General examination.
(3) Local chest examination.
(4) Routine laboratory investigations in the form of:
    (a) Complete blood picture.
    (b) Erythrocyte sedimentation rate.
    (c) Coagulation profile.
    (d) Fasting and 2 h postprandial blood glucose.
    (e) Liver function tests.
    (f) Kidney function tests: to exclude renal failure.
(5) Radiological examination including plain chest radiography posteroanterior and lateral views and computed tomography (CT) scans of the chest.
(6) Sputum examination for acid-fast alcohol fast bacilli by Ziehl–Neelsen stain.
(7) Tuberculin skin test [20]: using the Mantoux method where 0.1 ml (5 tuberculin units) of purified protein derivative was injected intradermally into the volar aspect of the forearm, then the test was read 48–72 h later, the reaction was considered positive if indurations of 10 mm or more in diameter were detected.
(8) Diagnostic thoracocentesis: the obtained pleural fluid (about 300–500 ml) was subjected to the following examinations:
    (a) Physical examination, including color, aspect, turbidity, and specific gravity.
    (b) Chemical examination, including proteins, glucose, and lactate dehydrogenase (LDH), where effusions were classified into exudates or transudates according to Light's criteria. If the effusion had any of the following three properties, the effusion was classified into exudates:
        (i) A ratio of the concentration of total proteins in pleural fluid to serum total proteins of more than 0.5.
        (ii) An absolute value of LDH of more than 200 IU.
        (iii) A ratio of pleural fluid LDH to serum LDH of more than 0.6 [20].
    (c) Bacteriological examination: including culture and sensitivity, pleural fluid examination for Gram stain, to exclude infectious causes other than tuberculosis,

and for acid-fast alcohol fast bacilli to detect tuberculosis bacilli by direct smear examination.

(d) Cytological examination: using the Papanicolaou-stained smears, hematoxylin and eosin-stained section of paraffin-embedded cell blocks.

(9) Pleural biopsies: were taken for all patients in groups (I, II) by either of the following methods: closed pleural biopsies using Abram's needle or thoracoscopic biopsies.

(10) Assay of the soluble calprotectin level in the pleural fluid by enzyme-linked immunosorbent assay: the principle of the test; the kit used double-antibody sandwich enzyme-linked immunosorbent one-step process assay to assay the level of calprotectin in pleural effusion. Add standard, the test sample and horseradish peroxidase-labeled calprotectin in pleural effusion antibodies to enzyme wells which are precoated with calprotectin in pleural effusion antibody, then incubation was carried out and washed to remove the uncombined enzyme. Upon adding chromogen solutions A and B, the color of the liquid changed to blue, and the reaction with the acid caused the color to become yellow. The depth of color and the concentration of the calprotectin in pleural effusion sample were positively correlated.

Statistical analysis [21]

The collected data were tabulated and analyzed using SPSS, version 16 software (SPSS Inc. Released 2007, SPSS for Windows, version 16.0; SPSS Inc., Chicago, Illinois, USA). Categorical data were presented as number and percentages, using χ^2 test to analyze them. Quantitative data were expressed as a mean ±SD, median, interquartile range. They tested for normality using the Shapiro–Wilk test, assuming normality at a *P* value of more than 0.05, Student's *t* test, and analysis of variance for normally distributed variables. Nonparametric variables were analyzed using the Mann–Whitney, Wilcoxon test, and Kruskal–Wallis test. Receiver operating characteristic curve was used to detect the cutoff value of calprotectin in the prediction of MPE.

The degree of significance in this work started below 0.05 ($P<0.05$ was considered significant). A *P* value of more than 0.05 is nonsignificant (NS), *P* value less than 0.05 is significant (S), and a *P* value less than or equal to 0.001 is highly significant (HS).

Results

This study included 60 patients: 26 (43.3%) men and 34 (56.4%) women. There were 14 (46.7%) men and 16 (53.3%) women in group I (malignant effusions) and there were 16 (53%) men and 14 (47%) women in group II (infectious effusions) which were subdivided into group IIA: the parapneumonic effusion group had 10 (67 %) men and five (33 %) women while group IIB included six (40%) men and nine (60%) women with tuberculous pleural effusion without significant difference between them.

The ages of the studied patients ranged from 19 to 76 years: group I (malignant effusions) with a mean age of 57.6±13 while group II (infectious effusions) was subdivided into group IIA, parapneumonic pleural effusion (their ages ranged 29–75 years with a mean 52±14) and group IIB, tuberculous pleural effusion (their ages ranged 13–65 years with a mean 44.8 ±14.5) with a significant difference between tuberculous and MPE.

The pleural calprotectin in group I MPE (229.2 ±168.6 ng/ml) was significantly lower than its level in group II; infectious pleural effusion was (3202.2 ±1304.8 ng/ml) with highly significant difference between two groups (Table 1).

The patients suffered from parapneumonic pleural effusions, group IIA demonstrated the highest levels of calprotectin (3333 ng/ml) and its level in patients with tuberculous pleural effusions group IIB (3071.3 ng/ml) were significantly higher than those of MPE group I (229.2 ng/ml), *P* value less than 0.001 (HS) (Table 2). However, the pleural calprotectin level in parapneumonic pleural effusion group IIA (3333±1151.8 ng/ml) was not substantially different from those in the tuberculous pleural effusion group IIB (3071.3 ng/ml) (P=0.92) (Table 3 and Fig. 1).

Table 1 Statistical analysis of pleural calprotectin level among the two studied groups

Effusion	*N*	Pleural calprotectin			MWU	*P*
		Mean±SD	Min	Max		
Malignant	30	229.2±168.6	99.0	1040.0	6.65	<0.001 (HS)
Nonmalignant	30	3202.2±1304.8	1040.0	5400.0		

HS, highly significant; Max, maximum; Min, minimum; MWU, Mann–Whitney *U* test.

Table 2 Statistical analysis of pleural calprotectin level among the studied subgroup

Groups	*N*	Pleural calprotectin			KWT	*P*	Significant pairs
		Mean±SD	Minimum	Maximum			
Malignant	30	229.2±168.6	99.0	1040.0	44.1	<0.001 (HS)	TB≠Malignant Parapneu.≠malignant
Parapneumonic	15	3333.0±1151.8	1040.0	5333.0			
Tuberculous	15	3071.3±1470.8	1145.0	5400.0			

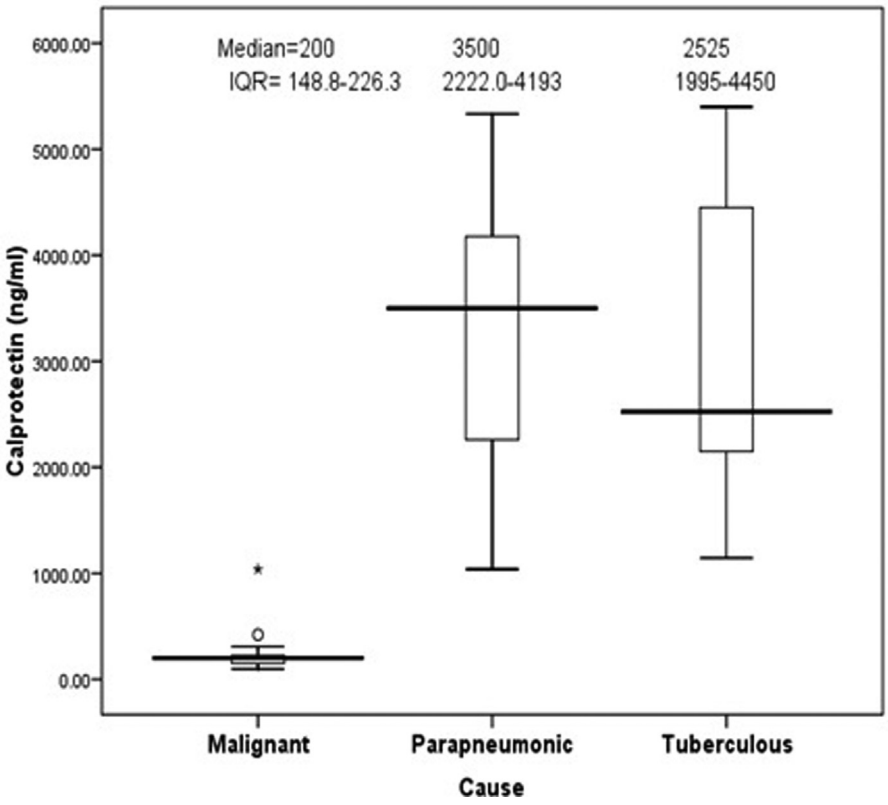
HS, highly significant; KWT, Kruskal–Wallis test; TB, tuberculosis.

Table 3 Statistical analysis of pleural calprotectin level in tuberculous and parapneumonic groups

Effusion	*N*	Pleural calprotectin			MWU	*P*
		Mean±SD	Min	Max		
Tuberculous	15	3071.3±1470.8	1145.0	5400.0	0.104	0.92 (NS)
Parapneumonic	15	3333.0±1151.8	1040.0	5333.0		

Max, maximum; Min, minimum; MWU, Mann–Whitney *U* test.

Figure 1



Box plot showing the median and the interquartile range (IQR) of pleural calprotectin.

Table 4 Cutoff value, sensitivity, specificity, and positive predictive value of pleural calprotectin level in the prediction of malignant pleural effusion

Variables	Cutoff	Sens%	Spec%	PPV%	NPV%	Accuracy%	AUC	95% CI	*P*
Pleural calprotectin	≤730.5	96.7	100	100	96.8	98.3	0.999	0.99–1.0	<0.001 (HS)

AUC, area under the curve; CI, confidence interval; HS, highly significant; NPV, negative predictive value; PPV, positive predictive value; Sens, sensitivity; Spec, specificity.

The cutoff value of calprotectin level for the diagnosis of MPE was less than or equal to 730.5 ng/ml; therefore, patients with calprotectin concentrations lower than this threshold had a high possibility of being diagnosed as MPE. The value for the area under the corresponding receiver operating characteristic curve [area under the curve (AUC)] was 0.999, the 95% confidence interval was 0.91–1 with 96.7% sensitivity and 100% specificity ($P<0.001$) (Table 4).

Discussion

Diagnosis of pleural effusion remains a challenge and the more important difficulty in the diagnosis of exudative effusion is to differentiate benign from malignant effusion [1].

This current study was carried out on 60 patients with exudative pleural effusion [group I with the mean age of 57.6±13 and group II (tuberculous with mean age 44.8±14.5, parapneumonic with mean age 52±14)].

These results are in accordance with Luo *et al.* [22] who studied 95 patients with pleural effusion; their mean age was as follows; malignant group 61±13.1; infectious group 47±20.6 (tuberculous 45±20.6). Sanchez-Otero *et al.* [18] also carried out a study on 156 patients, with 67 patients in the MPE group (men and 25 women, 42.9%) 42 and 89 patients in the BPE group (57.05%), where patients with BPE were secondary to tuberculosis (n=30, 20 men, 10 women) and parapneumonic (n=29, 22 men, seven women) and their mean age was 67.00±13.9 in MPE and 53±20 in BPE.

The pleural calprotectin in MPE (229.2±168.6 ng/ml) was significantly lower than its level in infectious pleural effusion (3202.2±1304.8 ng/ml) (Table 1). Sanchez-Otero *et al.* [18] had studied the concentration of pleural fluid calprotectin in the different diagnostic categories. The mean level of calprotectin in patients with MPE (257.2 ng /ml) was significantly lower than those with BPE (2627.1 ng/ml). Kohmo *et al.* [23] compared calprotectin and CXCL12 in MPE with that in BPE and demonstrated that calprotectin and the CXCL12 level significantly increased in nonmalignant pleural fluid compared with malignant pleural fluid, which supported the measurement of both biomarkers in pleural effusion as a possible noninvasive strategy for the differential diagnosis of MPE. Also Lou *et al.* [22] found that the median level of calprotectin and CXCL12 in MPE were 447.15 and 4.12 ng/ml, and both were significantly lower than that of the BPE (P=0.003 and 0.020, respectively) and that of tuberculous pleural effusion (P=0.002 and 0.003, respectively). The higher calprotectin levels found in BPE could attribute to the antimicrobial role of this protein [17].

Calprotectin level in patients with parapneumonic and tuberculous pleural effusions were significantly higher than those with MPE [3333, 3071.3, and 229.2 ng/ml, respectively with P<0.001 (HS)] (Table 2). However, the pleural calprotectin level in parapneumonic pleural effusion was not significantly different from those in the tuberculous pleural effusion (P=0.92) (Table 3). Sanchez-Otero *et al.* [18] found that patients with pneumonia showed the highest calprotectin level (3517.9 ng/ml) and were significantly higher than those of the MPF group (257.2 ng/ml), but these levels were not considerably different from those in the tuberculous group (2982.3±1573.0 ng/ml).

In the present work, the cutoff value of calprotectin level for the diagnosis of MPE was less than or equal to 730.5 ng/ml with 95% confidence interval and the AUC was 0.999; the corresponding sensitivity was 96.7% and specificity was 100% (P<0.001) (Table 4). Sanchez-Otero and colleagues detected the ability of calprotectin to differentiate between MPE and BPE by initiating two cutoff points. The first one (≤545 ng/ml) was determined as it gave the highest accuracy level (92.31%), and a sensitivity of 97.01% and specificity of 88.76%, while the second one (≤736.4 ng/ml) represented a sensitivity of 100%, specificity of 83.1%, accuracy of 90.4%, and AUC was found to be 0.963 (95% confidence interval, 0.932–0.994). Besides, they analyzed the calprotectin capability to differentiate pleural effusion subtypes. It showed the highest accuracy in differentiating MPE from pleural effusion of tuberculous and parapneumonic origin (97.94 and 95.83%, respectively) [18]. Luo *et al.* [22] stated that calprotectin showed high specificity in discriminating MPE from BPE and tuberculous pleural effusion (71.43 and 84.09%, respectively). On the contrary, Su and colleagues found a strong expression of S100-A8, S100-A9 in lung adenocarcinoma and end-stage lung cancer tissue. Also, Blanco-Prieto and colleagues found a similar level of calprotectin in the sera of lung cancer patients (221.21 ng/ml) but a lower serum level was found in benign inflammatory conditions (141.93 ng/ml). They explained that finding by the different nature of the fluids (pleural vs. serum) and their higher expression in lung tissues may be explained by the combined effect of inflammation on cancer progression [24,25].

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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