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Research Article

Seeking Biomarkers in Differential Diagnosis of Pleural Effusion: the Contribution of Measurements of Glycosaminoglycan, Cathepsin s, Cathepsin H and Vascular Endothelial Growth Factor

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Abstract

Background and Objectives

Pleural effusion is a frequently encountered problem in clinical practice. Cathepsin S and cathepsin H are produced by mesothelial cells located in the pleural cavity and play a role in the activation of acquired immune response. Vascular endothelial growth factor (VEGF) and glycosaminoglycans (GAGs) are known as the indicators of local inflammation and pathological development.

Methods

In this study, cathepsin S, cathepsin H, GAGs and VEGF were investigated along with other parameters for the diagnosis of effusion in 90 patients with pleural effusion in the outpatient ward of our department.

Results

There was a histopathologically confirmed distinction between benign and malignant pleurisy, in terms of pleural fluid/serum (PF/S) cathepsin S, PF cathepsin H, PF/S cathepsin H, PF VEGF and PF/S VEGF ($p=0.033$, $p=0.001$, $p=0.016$, $p=0.014$, and $p=0.015$, respectively). Biochemically, there was a significant difference in terms of S cathepsin S, PF cathepsin H, PF/S cathepsin H, PF VEGF, PF/S VEGF, PF GAGs ($p=0.037$, $p=0.008$, $p=0.009$, $p<0.001$, $p<0.001$, and $p=0.016$, respectively) between transudate and exudate. The difference between malignant and infectious effusion in regard to PF/S cathepsin S, PF cathepsin H parameters ($p=0.028$, $p=0.020$, respectively) was significant.

Conclusion

It was deduced that VGEF, cathepsin S, cathepsin H levels measured from effusion might be helpful in diagnosing malignant pleurisy while VEGF, cathepsin S, cathepsin H and GAG levels might be useful in diagnosing exudate.

Keywords: Acute-Phase Reaction,; Biological Markers; Inflammation; Pleura; Pleurisy

Introduction

The pleural effusion is one of the common problems encountered in clinical practice, and it has been reported that the patients with pleural effusion represent 4% of all admissions to the clinics of internal medicine. The rate of occurrence of pleural effusion is 4/1000 per year in general population [1]. The pleural effusion may result from pleural inflammation or malignancies as well as it may occur due to systemic disorders. For this reason, a differential diagnosis made promptly is of great importance for the treatment. The current parameters used for making the diagnosis are sometimes unsatisfactory and a diagnostic dilemma may be experienced[2]. With the studies conducted, novel parameters that can be used are being sought.

The cathepsin S and cathepsin H are molecules that play a role in the activation of the acquired immune response in the cells and tissues of immune system [2-4]. Vascular endothelial growth factor (VEGF) is a glycoprotein which increases permeability in the vascular endothelial cells and possesses angiogenic features, and it is released by many normal cells, tumor cells, and inflammatory cells [5]. The glycosaminoglycans (GAGs) are composed of chondroitin sulfate (chondroitin-4-sulfate and chondroitin-6-sulfate), heparan sulfate (heparan sulfate and heparin), dermatan sulfate, keratan sulfate, and hyaluronic acid [6]. The aforementioned biomarkers are produced by the mesothelial cells located in the pleural cavity [7].

These biomarkers were choosen because they are a current focus of research and same data suggest that they might be useful in differentiation of exudate or transudate and benign or malign.

In this study, we researched the value of cathepsin S, cathepsin H, glycosaminoglycan and vascular endothelial growth factor levels in pleural fluids, and the distinction between exudate/transudate, and between malignant/benign.

Materials and Method

The study included 90 patients who applied to the outpatient clinic of the Department of Pulmonary Medicine in the period between January 2010 and January 2011 and were diagnosed with pleural effusion depending on chest x-ray and pleural puncture. Of the patients included in the study, 30 had pleural effusions of hemodynamic origin, 30 had malignant effusions, and 30 had pleural effusions of inflammatory origin.

For our study, the ethics committee permission (dated 11/01/2010 with registration number of 2010/8) was obtained from the Mersin University Mersin No.1 Ethics Committee for Clinical Trials. All patients were informed and they provided written consents to participate in the study.

After clinical and radiologic evaluations, thoracentesis was performed on the patients who participated in the study. 50 ml of pleural fluid specimens and 20 ml of whole blood samples were collected concurrently from all the patients included in the study. The pleural fluid and blood specimens were analyzed for routine parameters, i.e. glucose, LDH, protein, albumin, cholesterol levels, and pH values. The supplementary albumin gradient was also used to distinguish transudate from exudate for the patients who had transudates that were presumed clinically and according to Light's criteria [1]. In addition, cell counts, Gram staining, and non-specific culture were done, and the specimens were examined for tuberculosis bacilli using Ziehl-Neelsen staining and mycobacteria growth indicator tube method and were also sent to the pathology laboratory for cytological examinations.

The pleural fluid and blood specimens put into anticoagulant-free tubes were centrifuged at 4000 rpm for 10 minutes, after which the sera were separated. The serum and pleural fluid specimens were allocated into individual Eppendorf tubes for every parameter and stored at -70°C until analysis.

The diagnoses of the patients with parapneumonic effusion were made depending on the presence of symptoms and clinical signs (fever, chills, shaking, purulent sputum), the presence of pneumonic infiltration on the chest x-ray on the same side where there was fluid accumulation, and the response to the antimicrobial therapy. The diagnosis of tuberculosis pleurisy was established based on the detection of tuberculosis bacilli on the pleural fluid specimen and/or growth of bacilli on the culture of pleural fluid and/or detection of caseating granulomatous inflammation on the pleural biopsy specimen. The diagnosis of congestive heart failure was made based on enlarged cardiac silhouette on chest x-ray, recognition of heart failure on echocardiography, larger jugular veins, peripheral edema, and ventricular gallop. The diagnosis of malignant pleural effusion was established by the demonstration of malignancy histologically or cytologically on pleural fluid, biopsy specimen, or with VATS, and primary focus was investigated.

Study patients were tested for cathepsin S, cathepsin H and VEGF levels ELISA GAG levels along with the parameters such as protein, glucose, pH, albumin, lactate dehydrogenase (LDH),

cholesterol, triglyceride, C-reactive protein (CRP), amylase.

The cathepsin S level was measured with ELISA assay using Uscn Enzyme-linked Immunosorbent Assay Kit for human cathepsin S (CTSS) (Uscn Life Science Inc. China, Catalog no: E91933Hu). The cathepsin H level was measured with ELISA assay using Cusabio Human Cathepsin H (CTSH) ELISA Kit (CUSABIO BIOTECH Co., Ltd., China, Catalog No: CSB-E13723h). The VEGF level was determined by ELISA assay using RayBio Human VEGF ELISA (RayBiotech, Inc., Georgia, USA, Catalog no: ELH-VEGF-001) kit. An ELISA plate with anti-VEGF antibody-coated wells was used. Glycosaminoglycan levels were determined using Kamiya Biomedical Company GAGs Assay (KAMIYA Biomedical Company, Washington, USA, Cat. No. BP-004) kit.

Statistical Analysis

Given the assessment of the data obtained, SPSS (Statistical Package for Social Sciences) for Windows 11.5 software was used for statistical analysis. First, the compliance of continuous variables with normal distribution was assessed using Shapiro Wilk test, thereafter; it was determined that non-parametric methods should be used. Mann Whitney U test was used for analyzing numeric variables. Chi-square test was used for analyzing categorical variables. For the parameters possessing significant distinctive values, the cut-off values were determined with Roc analysis, and sensitivity and specificity statistics were calculated. The value of statistical significance was set to be 0,05.

Results

The diagnoses of the groups of patients are presented in table 1.

Table 1. The diagnoses of the groups of patients included in the study.

Group of patients	Disease type	Number of patients n=90
Hemodynamic origin	Congestive Heart Failure	n= 30
Malignant origin	Malignant mesothelioma	n= 5
	Small cell lung carcinoma	n= 4
	Non-small cell lung carcinoma	n= 11
	Breast carcinoma	n= 2
	Gastric carcinoma	n= 4
	Colon carcinoma	n= 3
	Ovarian carcinoma	n= 1
Infectious origin	Pneumonia	n= 19
	Tuberculosis	n= 11

The median age of the study patients was 59.6±15.8. Age distribution by the type of disorder is presented in table 2. Of 90 patients included in the study, 54 (60%) were men and 36 (40%) were women.

Table 2. Age distribution by the type of disorder.

Parameter	n	Minimum	Maximum	Mean	Standard Deviation
Type of Disorder					
Malignant	30	29.00	83.00	60.83	12.04
CHF*	30	36.00	93.00	65.06	13.37
Infection	30	21.00	84.00	52.93	19.24
Type of path.					
Benign	60	21.00	93.00	59.00	17.53
Malign	30	29.00	83.00	60.83	12.04
Type of chem.					
Transudate	30	36.00	93.00	65.06	13.37
Exudate	60	21.00	84.00	56.88	16.39

*CHF: Congestive Heart Failure

Table 3. The statistical analyze of biochemical parameters in distinction of benign and malignant conditions.

Biochemical parameters	Benign	Malign	P value
PF/S katepsin S	0.66 ± 0.72	2.81 ± 9.56	p=0.033
PF katepsin H(ng/ml)	25.88 ± 8.57	11.78 ± 26.22	p=0.001
PF/S katepsin H	0.68 ± 1.15	2.93 ± 6.02	p=0.016
PF VEGF(pg/ml)	2805.09 ± 5955.33	10103.55 ± 17890.83	p=0.014
PF/S VEGF	8.93 ± 23.69	12.68 ± 22.64	p=0.015

PF: Pleural fluid, S: Serum, VEGF: Vascular endothelial growth factor,

There was a statistically significant difference between benign and malignant disease groups with regards to PF/S (pleural fluid/serum) cathepsin S, PF (pleural fluid) cathepsin H, PF/S cathepsin H, PF VEGF and PF/S VEGF (p=0.033, p=0.001, p=0.016, p=0.014,and p=0.015, respectively, Table 3).

There was a statistically significant difference between the groups of transudates and exudates with regards to S (serum) cathepsin S, PF cathepsin H, PF/S cathepsin H, PF VEGF, PF/S VEGF, and PF GAGs (p=0.037, p=0.008, p=0.009, p<0.001, p<0.001, and p=0.016, respectively, Table 4).

PF/S cathepsin S levels were higher in the group of malignant effusions (2.81±9.55) in comparison with the group of infections (0.57 ± 0.58) (p=0.028). PF cathepsin H levels were also higher in the group of malignant effusions (11.78 ± 26.22) in comparison with the group of infections (3.05 ± 10.49) (p=0.020).

Table 4. The statistical analyze of biochemical parameters in distinction of transudates and exudates.

Biochemical parameters	Transudate	Exudate	P value
S katepsin S (ng/ml)	86.02 ± 68.42	59.32 ± 46.43	p=0.037
PF katepsin H (ng/ml)	2.12 ± 6.22	7.42 ± 20.28	p=0.008
PF/S katepsin H	0.44 ± 0.27	1.93 ± 4.48	p=0.009
PF VEGF (pg/ml)	1200.77 ± 5571.11	7256.48 ± 13533.83	p<0.001
PF/S VEGF	1.88 ± 7.37	14.33± 27.17	p<0.001
PF GAGs (µg/ml)	95.31 ± 61.26	79.24 ± 54.80	p=0.016

PF: Pleural fluid, S: Serum, VEGF: Vascular endothelial growth factor,

GAGs: glycosaminoglycans

Table 5. The criteria for distinguishing malignant from benign, which were determined with ROC analyze.

Biochemical parameters	Cut Point	Under curve area	95%CI	p*
PF/S katepsin S	>0.73	0.639	0.53-0,738	0.0347
PF katepsin H	>1.426	0.711	0.61-0.81	0.0008
PF/S katepsin H	>0.96	0.657	0.55-0.75	0.0228
PF VEGF	>2731.66	0.66	0.55-0.76	0.0122
PF/S VEGF	>1.11	0.66	0.55-0.76	0.0107

*Under curve area p value , PF: Pleural fluid, S: Serum, VEGF: Vascular endothelial growth factor

The ROC analysis was done to determine the distinctive values of the parameters found to be significant in our study. Five parameters were found to be significant in distinction of benign and malignant conditions (Table 5).

For PF/S cathepsin S, the critical value in distinction of benign and malignant conditions was 0.73. The sensitivity corresponding the critical value was 63.3 (43.9–80.1) and the specificity was 71.19 (57.9–82.2) (p<0.05). For PF cathepsin H, the critical value in distinction of benign and malignant conditions was 1.426. The sensitivity corresponding the critical value was 53.3 (34.3–71.7) and the specificity was 86.44 (75.0–94.0). The test has a strong ability to distinguish benign conditions. For PF/S cathepsin H, the critical value in distinction of benign and malignant conditions was 0.96. The sensitivity corresponding the critical value was 46.67(28.3–65.7) and the specificity was 91.53(81.3–97.2). The test has a strong ability to distinguish benign conditions.

For PF VEGF, the critical value in distinction of benign and malignant conditions was 2731, 65. The sensitivity corresponding the critical value was 46.7 (28.3–65.7) and the specificity was 81.4 (69.1–90.3). The test has a strong ability to distinguish benign conditions. For PF/S VEGF, the critical value in distinction of benign and malignant conditions was 1.11. The sensitivity corresponding the critical value was 70 (50.6 – 85.3) and the specificity was 64.4 (50.9 – 76.4). The test has a strong ability to distinguish malignant conditions.

As a result of logistic regression analysis, serum levels of VEGF, cathepsin S, cathepsin H and GAGs were found to be insignificant to separate benign and malignant conditions. However, PF levels of both VEGF and cathepsin individually were found to be significant in distinction between benign and malignant conditions. If these four parameters are evaluated together, VEGF is the most significant one (p=0.036). It is followed by GAG, cathepsin H and cathepsin S. However, these parameters were statistically insignificant. Given PF/S ratios, cathepsin S, cathepsin H, and VEGF were significant alone, but not so when evaluated together (p>0.05).

As a result of logistic regression analysis done to distinguish transudates from exudates, serum cathepsin S alone was distinctive for distinguishing transudates from exudates, and it was found to be the most significant parameter when they were evaluated together (p=0.043). Pleural fluid cathepsin H, VEGF and GAG were distinctive alone for distinguishing transudates from exudates, and VEGF was found to be the most significant parameter when they were evaluated together (p=0.044). Given PF/S ratio, cathepsin H and VEGF alone were distinctive, and cathepsin H was found to be the most significant parameter when they were evaluated together (p=0.016).

Discussion

In this trial, we studied the contribution of VEGF, cathepsin S, cathepsin H, and GAG to etiologic diagnosis of pleural fluid not only in distinction between malignant and benign effusion, but also in distinction of exudates using transudates of the control group patients with CHF. We paid attention to form patient groups that were identical with regards to age and gender distributions; in-group comparisons were done and the groups were compared to each other; and the power of interpretations was increased by using multiple analysis methods. Finally, the contribution of the analysis to the diagnosis and the sensitivities of the methods were calculated individually and compared with the literature data.

Cathepsin S and cathepsin H exist in immune cells and tissues, and activate the adaptive immune response. They are involved in degradation of extracellular matrix and are related to both inflammatory and neoplastic processes. Therefore, it is expected that they would increase in exudative pleural effusions [8-11]. Among previous studies, there are conflicting reports that either support that the levels of cathepsin S and cathepsin H might be helpful to distinguish effusions with different etiologies, or find this insignificant [12,13]. In our study, cathepsin S and cathepsin H were found to be significant in the distinction between benign and malignant effusions, as well as between transudates and exudates, and between malignant and infectious effusions. In our study, specific ratios were determined by comparing the values found in all measurements with serum levels, and this situation was considered in a statistical assessment. Moreover,

the measurements done in transudates obtained from CHF patients in the control group strengthened the interpretations. As a biomarker, PF/S cathepsin S measurement appears to be a significant marker for the diagnosis of particularly malignant pleural effusion.

VEGF is a multifunctional cytokine, which plays a role in both pathological and physiological angiogenesis as well as in lymphangiogenesis. Several pathological conditions other than malignancies, hypoxia, pulmonary embolism, hemorrhagic effusions, and empyema may cause an increase in VEGF levels [14-16]. That VEGF level increases in pleural effusions and serum in malignant processes suggests that VEGF can be used as a marker in malignant conditions [17-23] However, there are reports defining low levels of VEGF measured in malignant pleural effusions [24,25].

Although there are many studies about VEGF in the literature, it has been evaluated in a limited number of studies in distinction between benign and malignant conditions. A meta-analysis study published by Shen et al. in 2012 included 181 trials about VEGF, but only 10 of these had a control group to establish a distinction between benign and malignant conditions This meta-analysis evaluating 514 malignant pleural effusions and 511 benign pleural effusions found a moderate sensitivity of 0.75 (95% CI 0.72-0.79) and a specificity of 0.72 (95% CI 0.68-0.76) for VEGF. Considering this result, it was concluded that VEGF might play a role in the diagnosis of malignant pleural effusions if it was used together with clinical findings and conventional methods [26].

In our study, a significant difference was found with regards to PF VEGF and PF/S VEGF in distinction between malignant and benign pleural effusions. VEGF was not associated with age, gender, and smoking. Whilst PF VEGF measurements alone had a sensitivity of 46.7% and a specificity of 81.4% in differential diagnosis of malignant pleural effusions, these rates rose to 70% and 64.4%, respectively, if pleural fluid measurements were proportionated to serum levels (PF/S). The diagnostic power of this method is at the statistically significance level in both conditions ($p<0.05$). To the best of our knowledge, this is the first trial which defined VEGF levels that were measured in distinct types of pleural fluids in proportion to its serum levels, and included comparisons between different types of effusions.

There are reports supporting the role of VEGF measurements in distinction between the transudative and exudative pleural fluids [27,28]. Our study also endorses this opinion. However, its routine use is questionable in terms of cost-effectiveness, considering that high expenditure is not proportional to the matter of distinction between transudate and exudate. This is a relatively less troublesome situation in contrast to the distinction between malignant and benign processes.

In most of the pleurisy, glycosaminoglycans were found in the pleural fluid. In benign and malignant pleural effusions, serum GAG was found to be a marker with the best sensitivity and specificity scores [2,7,29-31]. LDH, a classical parameter among Light's criteria, has slightly higher sensitivity than PF GAGs (88.9% for LDH vs. 86.7% for PGAGs), but its specificity was found to be lower than PF GAGs (80% for LDH vs. 100% for PGAGs). In our study, there was a significant difference between the groups of transudates and exudates with regards to PF GAGs ($p=0.016$). However, GAGs measurements did not contribute significantly to the distinction between benign and malignant pleural fluids.

This study has some restrictions. A trial with larger sampling size, including sham controls, and with long-term follow up could have provided more information. However, there were not such possibilities because of both ethics committee regulations and financial constraints. However, our trial is noteworthy since it is the first clinical study in the literature, which investigated the diagnostic values of cathepsin S, cathepsin H, VEGF and GAGs as biomarkers in the diagnosis of either malignant or infectious pleural effusion in a cross-sectional case-control model and compared PF and serum measurements.

Conclusion

-PF/S cathepsin S and PF cathepsin H measurements are useful to diagnose malignant and exudative pleural effusions. PF/S VEGF measurements are preferred to diagnose malignant and exudative pleural effusions. PF GAGs measurement is helpful to diagnose exudates, however it does not contribute significantly to the diagnosis of malignant pleural effusions.

Authors Contributions

NG, CÖ, GP, BT and ESÖ designed the study, drafted and corrected the manuscript; NG, CÖ, GP, SA, ESÖ, SN and Aİ collected the study data; NG, CÖ, BT and SN interpreted the study data; NG, CÖ, GP, SA, BT, ESÖ, SN and Aİ revised critically the manuscript and approved its final version.

Conflicts of İnterest

The authors have disclosed no conflicts of interest.

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