RESEARCH COMMUNICATION

Application of MMP-7 and MMP-10 in Assisting the Diagnosis of Malignant Pleural Effusion

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Abstract

Background: Matrix metalloproteinases (MMP) are proteolytic enzymes that are essentially involved in turnover of the extracellular matrix (ECM). The aim was to investigate the diagnostic value of MMP-7 and MMP-10 as tumor markers in pleural effusion (PE) and evaluate the value of combining MMP-7, MMP-10 and carcinoembryonic antigen (CEA) assays as diagnostic aids for malignant cells. Materials and Methods: A total of 179 patients with PE (87 malignant and 92 benign) were included in this study. The levels of MMP-7 and MMP-10 were measured using ELISA. Results: Values for MMP-7 and MMP-10 were significantly higher in malignant PE than those in benign PE (P<0.01). Among all variables evaluated, logistic regression found that MMP-7 and MMP-10 were significantly correlated with the presence of malignant disease (P<0.01). Analysis of receiver operating characteristics (ROC) curves showed that the area under the curve of MMP-10 (0.806) was significantly larger than that of MMP-7 (0.771) and CEA (0.789) (P<0.01). With parallel interpretation, the combination of MMP-10 and CEA achieved the higher sensitivity of 94.6%. The combination of MMP-7 and CEA in serial interpretation was able to boost the specificity to 95.7%. The combination of MMP-7, MMP-10 and CEA produced better sensitivity, specificity, PPV and NPV than MMP-7 and MMP-10 alone. Conclusion: MMP-7 and MMP-10 in PE may represent helpful adjuncts to conventional diagnostic tools in ruling out malignancy as a probable diagnosis, thus guiding the selection of patients who might benefit from further invasive procedures.

Keywords: Matrix metalloproteinases - pleural effusion - diagnosis - cancer

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Introduction

Pleural effusion (PE) are defined as accumulations of free liquid in the pleural space caused by increased production or decreased clearance of pleural fluid (Sahn, 1988; Miserocchi, 1997). PE is a commonly occurring complication produced by a wide variety of diseases, such as tumor, tuberculosis, congestive heart failure, and pneumonia, et al. Approximately 20% of pleural effusions are caused by malignancy, and, in 10 to 50% of cancer patients, may be the initial presentation (Monte et al., 1987). Establishing the diagnosis of a malignant pleural effusion indicates advanced disease and is associated with limited survival (van de Molengraft et al., 1989). It is then clinically important to clarify the precise cause of the PE, especially to discriminate benign from malignant effusions.

To date, patients with PE frequently present a diagnostic challenge. Definitive proof of malignant pleural effusion requires invasive thoracentesis examination. However, this procedure is uncomfortable and lab-intensive, particularly in follow-up. Although cytology is widely used for screening for malignant PE, it is sometimes difficult to judge cytologic specimens, particularly for low-grade cancers. The sensitivities of biochemical parameters and tumor markers of PE are often unsatisfactory (Rittgers et al, 1978; Fenton et al, 1995). Approximately 20% of patients with malignant pleural effusions still could not be confirmed (Fenton et al., 1995).

In search of a better method to diagnose malignant pleural effusions, the roles of matrix metalloproteinases (MMPs) are investigated. MMPs belong to the group of extracellular matrix (ECM) degradation enzymes (Ries et al., 1995), and constitute a family of 23 structurally related zinc-dependent endopeptidases engaged in various physiological processes, such as apoptosis, proliferation, differentiation, tumor angiogenesis, and malignant conversion by degrading ECM (Woessner, 1991). Previous studies have demonstrated that MMP-7 plays an important role in the metastases of endometrial, gastrointestinal carcinomas, colorectal cancer, and lung cancer (Shiomi et al., 2003; Safranek et al., 2009; Shi et al., 2010). It has been demonstrated that MMP-10 was overexpressed in several human tumors of epithelial origin, including gastric cancer, esophageal carcinoma, skin carcinoma, and bladder transitional cell carcinoma determined using the immunohistochemical staining or microarray techniques

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(Kerkela et al., 2001; Mathew et al., 2002; Seargent et al., 2005; Aung et al., 2006). There is no published study on the detection of MMP- 7 and MMP-10 in PE. The objective of this study was to determine the efficiency of MMP-7 and MMP-10 in the detection of the origin of PE and demonstrated these tests as potentially useful in assisting the diagnosis of malignant PE.

Materials and Methods

Patients

The study included 179 consecutive patients with PE who were recruited from the No.202 hospital in Shenyang from Feb, 2010 to Dec, 2010. All PE had definite etiology documented by examination of effusion biochemistry, cytology, pleural biopsy, percutaneous biopsy, endoscopic examination, and clinical follow-up. Malignant pleural effusion (n = 87) was confirmed when malignant cells were found in the effusion by cytological examinations or in the specimens taken from pleural needle biopsy or thoracoscopic biopsy. Of the 87 patients with malignant PE (61 males and 26 females, mean age, 48.1 ± 11.5 years), 28 were lung adenocarcinoma, 18 were squamous cell carcinomas, 6 were small cell lung cancer, 9 were pleural mesothelioma, 10 were breast cancer, 2 were thyroid cancer, 3 were gastric adenocarcinoma, 4 were nasopharyngeal carcinoma, 3 were lymphoma, and 4 were other cancers. Only specimens diagnosed as primary malignancies were included and otherwise were excluded. A total of 92 samples (64 males and 28 females, mean age, 45.5 ± 13.6 years) were from patients with benign diseases. Of the 92 patients with benign PE, there were 29 patients with tuberculous pleurisy, 22 patients with parapneumonic effusion, 25 patients with heart failure, 10 patients with hepatic hydrothorax, 3 patients with ovarian hyperstimulating syndrome, 3 patients with other diseases. Exclusion criteria included diabetes mellitus, existence of ascite, gastrointestinal bleeding, renal failure, treatment with corticosteroids, and immunosuppressive agents within the last 6 months.

Sample collection and biochemical analyses

Each sample of PE was collected in a syringe during a thoracentesis performed after written informed consent was obtained. Samples were centrifuged at 1500 × g for 10 min at -4°C. The supernatant were stored at -80°C for assessment of the levels of MMP-7, MMP-10 and carcinoembryonic antigen (CEA). The levels of MMP-7 and MMP-10 in PE were determined with enzyme-linked immunosorbent assay (ELISA) (R&D SYSTEMS, INC. USA). Concentration of CEA was measured by electrochemiluminescence kits (Roche Diagnostics, Beijing, China). All assays were run in duplicate, with dilutions as appropriate, and the technicians were blinded to clinical data.

Statistical analysis

Statistical evaluation was performed with SPSS Software 13.0 (SPSS Inc., Chicago, IL). Values were reported as the median (min-max) for non-normally distributed variables. The Mann-Whitney's U-test was used to assess the difference between benign and malignant groups and logistic regression to determinate the relationship between the presence of malignant and all variables. For the optimum cut-off point provided by receiver operating characteristic (ROC) curves analysis, sensitivity, specificity, positive predictive value (PPV), negative predictive values (NPV) and Youden's index (YI) were calculated. Combination tests were used to determine sensitivity, specificity, PPV, and NPV, respectively. A value of P<0.05 was considered statistically significant.

Table 1. Logistics Regression Analysis

Variables	OR	95%CI	Coefficient	SE	P value
MMP-7	1.004	1.001-1.007	0.004	0.002	0.003
MMP-10	1.015	1.009-1.021	0.015	0.003	0.000
CEA	1.768	1.334-2.342	0.570	0.114	0.000

Note: OR, odds ratio; CI, confidence interval; SE, standard error; MMP, matrix metalloproteinases; CEA, carcinoembryonic antigen

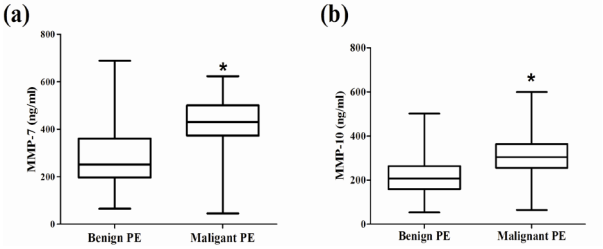

Figure 1. Levels of MMP-7 and MMP-10 in Pleural Effusion Between Benign and Malignant PE Groups. (a) MMP-7. (b) MMP-10. * indicates P<0.01 compared with benign PE group

Results

MMP-7 and MMP-10 levels in PE

With respect to gender and age, we did not find any significant difference between malignant and benign groups. As shown in Figure 1a, the level of MMP-7 was significantly higher in patients with malignant PE (431 ng/mL [46-624 ng/mL]) than in those with benign PE (252 ng/mL [65-589 ng/mL]) (P<0.01). Figure 1b showed that the level of MMP-10 was 302 ng/mL (65-601 ng/mL) in malignant PE, and it was significantly higher compared to the value of 205 ng/mL (54-502 ng/mL) found in benign PE (P<0.01).

Logistic regression analysis

For MMP-7, MMP-10 and CEA, all variables were significantly correlated with the presence of malignant diseases (P <0.001), shown in Table 1.

Diagnostics utility of MMP-7 and MMP-10

For a certain marker, a value less than 0.7 for the area under the receiver operating curves (AUROC) means that it is not possible by which to differentiate the two compared groups. AUROCs obtained from the analysis, performed in all patients, showed the following values: 0.771 (MMP-7), 0.806 (MMP-10) and 0.789 (CEA), as shown in Figure 2. The best cut-off values for investigated parameters were calculated by the ROC curves as 353 ng/mL for MMP-7, 243 ng/mL for MMP-10 and 2.65 μg/

Table 2. Diagnostic Values of MMP-7 and MMP-10 for Predicting Malignant PE

Variables	AUC (95%CI)	Accuracy (%)	Sensitivity (%)	Specificity (%)	Cut-off	PPV (%)	NPV (%)	YI
MMP-7	0.771(0.698-0.845)	78.2	82.8	73.9	353 ng/ml	75.0	82.0	0.567
MMP-10	0.806(0.740-0.873)	74.9	86.2	64.1	243 ng/ml	69.4	83.1	0.503
CEA	0.789(0.724-0.855)	72.1	60.9	83.6	8.25 μg/ml	77.8	69.3	0.445

Note: AUC, area under curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; YI, Youden's index

Table 3. Assessment of the Diagnostics Performance of Combining MMP-7, MMP-10 and CEA

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
MMP-7 ∪ CEA	93.3	61.8	69.8	90.7
MMP-10 ∪ CEA	94.6	53.6	65.8	91.3
MMP-7 ∩ CEA	50.4	95.7	91.7	67.1
MMP-10 ∩ CEA	52.5	94.1	89.4	67.7

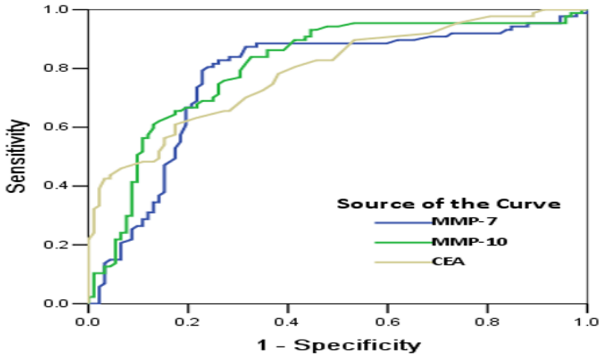

Figure 2. Receiver Operating Characteristic (ROC) Curves of MMP-7, MMP-10 and CEA for the Identification of Malignant PE

mL for CEA. Table 2 showed the sensitivity, specificity, negative predictive value (PPV), negative predictive value (NPV) and Youden's index of MMP-7, MMP-10 and CEA. MMP-10 achieved the the highest sensitivity (86.2%) followed by MMP-7 (82.8%) and CEA (60.9%). The specificity of MMP-7 (73.9%) and MMP-10 (64.1%) were lower than CEA (83.6%). The Youden's index of MMP-7 (0.567) was the highest among these markers.

Combined performance of all investigated markers

The combined performance of MMP-7, MMP-10 and CEA are shown in Table 3. With parallel interpretation, the diagnostic performances of MMP-7 ∪ CEA and MMP-10 ∪ CEA were significantly improved compared with using a single marker; the combination of MMP-10 ∩ CEA achieved the higher sensitivity of 94.6% and NPV of 91.3%. On the other hand, the combination of MMP-7 ∩ CEA in serial interpretation was able to boost the specificity to 95.7% and the PPV to 91.7%. The combination of MMP-7, MMP-10 and CEA produced better sensitivity, specificity, PPV and NPV than MMP-7 and MMP-10 alone.

Discussion

The definitive diagnosis of malignant PE is largely based on positive cytological and histological results, but the sensitivity is only about 60% (Dekker et al., 1976; Sahn, 1988). A large number of studies on the potential diagnostic value of pleural tumor markers have been published, which report either encouraging or disappointing results (Wobbes et al., 1992; Garcia-Pachon et al., 1997; Riantawan et al., 2000; Paganuzzi et al., 2001). The disagreement in results can be attributed to different factors, including the heterogeneity of tumor types, the appropriate inclusion of paramalignant PE to calculate test performances, the sometimes underpowered series, and the use of different methodologies and cut-off values in tumor marker assays (Porcel et al., 2004). CEA is the most commonly studied and used, with an accuracy in PE greater than that of other tumor markers (Romero et al., 1996). Nevertheless, in almost series on CEA in PE, high false positive results have been reported (Garcia-Pachon et al., 1997). Thus, it is necessary to find some markers with higher sensitivity and specificity in the diagnosis of malignant PE.

Malignant PE is a common occurrence in many patients with cancer. Typically, tumor cells invade the basement membrane by secreting enzymes that digest the extracellular matrix proteins. These enzymes are known as MMPs. MMP-7, named matrilysin, is expressed in lung cancer, colonic adenocarcinomas, urinary bladder cancer, gastric cancer, breast cancer, and other types of cancers (Newell et al., 1994; Liu et al., 2008; Beeghly-Fadiel et al., 2009; Shi et al., 2010; Szarvas et al., 2011). The clinical impact of its presence in cancer has been evaluated, and increases in its expression have been associated with the presence of lymph node and distant metastases. However, this enzyme seems to induce angiogenesis, thus stimulating the proliferation of vascular endothelial cells and the formation of new vessels adjacent to the tumor, which facilitates metastatic dissemination. MMP-10, named stromelysin-2, also has been detected in several carcinomas (Birkedal-Hansen et al., 2000; Bodey et al., 2001; Kerkela et al., 2001; Mathew et al., 2002). Gill reported that MMP-10 as a potential tumor marker and a predictor of clinical behavior, and supports MMP-10 inhibition as a valid therapeutic strategy. In agreement with previous reports, we found that the levels of MMP-7 and MMP-10 were significant higher in malignant PE than those in benign PE. The raised MMP-7 and MMP-10 levels in malignant PE may be related to increased ECM degradation and endothelial permeability. Considering MMP-7, MMP-10 and CEA, all variables were significantly correlated with the presence of malignant disease. Thus, in addition to its ability to ECM degradation, MMP-7 and MMP-10 maybe contribute to tumor growth and metastases.

Among PEs in patients with malignant tumor, 68/87 had MMP-7 levels > 353ng/mL, and 59/87 had MMP-10 levels >243 ng/mL, respectively. A possible explanation

for these data include: (1) the presence of a small number of tumor cells that secrete MMP-7 and MMP-10; (2) metastasis confirmed in the surface tissue, but secreting MMP-7 and MMP-10 into the pleural cavity; (3) cell death or destruction with a residual presence of MMP-7 and MMP-10 in PE. On the other hand, 19/87 had low levels of MMP-7, and 28/87 had low levels of MMP-10. First, expression of MMP-7 and MMP-10 is tissue specific, and not all types of cancer can secrete enough MMP-7 and MMP-10. Second, expression of MMP-7 and MMP-10 are different according to clinical grade and pathologic stage. Third, the balance of MMPs and their specific inhibitors (tissue inhibitor of metalloproteinases, TIMPs) play an important role in maintaining connective tissue homeostasis in normal tissue (Nagase et al., 1999; Nelson et al., 2000; Sounni et al., 2005). Overexpression of TIMP may result in down-regulation of MMPs levels (Wasenius et al., 2003).

The practical use of a tumor marker is linked to its capacity to detect occult disease at a still treatable stage or to reliably indentify occult metastases (Fiorelli et al., 2011). In ROC analysis MMP-7 levels provide a sensitivity and specificity value of 82.8% and 73.9%, and MMP-10 levels provide a sensitivity and specificity value of 86.2% and 64.1%, respectively. MMP-10 level achieved the largest AUROC in all variables. These results suggest a potential clinical usefulness of pretreatment MMP-7 and MMP-10 levels for the differentiation between malignant and benign PE. CEA, as a useful tumor marker, its sensitivity for the diagnosis of malignant PE was lower than those of MMP-7 and MMP-10, but specificity was higher than those of them. In our present study, compared with other studies, sensitivity and specificity were varied due to different cut-off values chosen. Yet to substantiate a diagnosis of malignant PE, cut-off values for a tumor marker that are 100% specific need to be chosen. If we adopt high cut-off levels that are not exceeded by none of the benign PE, although high specificity is reached, it makes tumor marker assay very insensitive. There was always a balance between sensitivity and specificity. The diagnostic performance of MMP-7 and MMP-10 levels increased with combining CEA tumor marker. The combination of MMP-10 $\cup$ CEA achieved the higher sensitivity of 94.6% and NPV of 91.3% in parallel interpretation. On the other hand, the combination of MMP-7 $\cap$ CEA in serial interpretation was able to boost the specificity to 95.7% and the PPV to 91.7%. The combination of MMP-7, MMP-10 and CEA produced better sensitivity, specificity, PPV and NPV than MMP-7, MMP-10 and CEA alone.

In conclusion, we found that the levels of MMP-7 and MMP-10 were higher in malignant PE than those in benign PE. Additionally, the levels of MMP-7 and MMP-10 correlated with the presence of malignant PE. Diagnostic sensitivity of MMP-10 was found to be the highest among all of the variables tested. Moreover, AUROC of MMP-10 was larger than those of either MMP-7 or CEA. Our results thus suggest the levels of MMP-7 and MMP-10 in PE are useful markers in differentiating malignant PE from benign PE.

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