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Diagnostic performance of CD66c in lung adenocarcinoma-associated malignant pleural effusion: comparison with CEA, CA 19-9, and CYFRA 21-1

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Summary

Various tumour markers have been evaluated in malignant pleural effusions, but not CD66c. This study evaluated the diagnostic ability of CD66c in lung adenocarcinoma-associated malignant pleural effusions (LA-MPEs) and compared it with other known tumour markers. Forty-seven cases of LA-MPE and 52 cases of benign pleural effusions were collected. The levels of CD66c, CEA, CA 19-9, and CYFRA 21-1 were measured by enzyme immunoassay. The expression of CD66c, CEA, and CA 19-9 in cell blocks was measured by immunocytochemistry. CEA had the best diagnostic values, with a sensitivity of 87.2% and specificity of 92.3%. Both CD66c and CA 19-9 showed the highest specificity of 98.1%, with sensitivities of 63.8% and 55.3%, respectively. CYFRA 21-1 had a sensitivity of 83.0% and specificity of 76.9%. CEA combined with CA 19-9 reached a sensitivity of 91.5% and a specificity of 98.1%. The sensitivities of immunocytochemical staining for CD66c, CEA, and CA 19-9 were 72.5%, 75%, and 40%, respectively. CD66c showed a diagnostic performance comparable to CYFRA 21-1 and CA 19-9 by enzyme immunoassay. Immunocytochemical study showed that CD66c and CEA were more sensitive than CA19-9. Both studies support CD66c as a potential tumour marker to differentiate LA-MPE from benign effusions.

Key words: CA 19, CD66c, CEA, CEACAM6, CYFRA 21, lung adenocarcinoma, pleural effusion.

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INTRODUCTION

Lung cancer is the leading cause of cancer death worldwide.[1] Malignant pleural effusions (MPEs) are common complications of lung cancer, especially adenocarcinoma. Approximately 15% of lung cancer patients have pleural effusion at the time of initial diagnosis, and up to 50% develop it later in the course of their disease.[2,3] The detection of MPE is important because this has a poor prognosis and is classified as stage IV disease. Cytological examination of pleural effusions obtained through thoracentesis is a standard and non-invasive method for the diagnosis of MPE; however, this method has a sensitivity of only 40–70%.[4] Consequently, the search for useful tumour markers to evaluate pleural effusions is continuing. Several markers, including carcinoembryonic antigen (CEA), cytokeratin fragment (CYFRA) 21-1, cancer antigen (CA) 19-9, CA 15-3, CA 125, CA 72-4, and tumour-associated glycoprotein (TAG) 72, are reported to have diagnostic significance.[5–15]

CD66c is a member of the CD66 antigen family encoded by the carcinoembryonic antigen-related cell adhesion molecule (CEACAM) genes located on chromosome 19q13.2. The glycoprotein products of CEACAM1, CEACAM8, CEACAM6, CEACAM3, and CEACAM5 are classified as CD66a, CD66b, CD66c, CD66d, and CD66e (CEA), respectively.[16,17] CD66c is a glycophosphatidylinositol (GPI)-anchored cell surface glycoprotein composed of an extracellular region containing three immunoglobulin-like domains.[18] It shares some antigenic determinants with CEA, but the expression level of CD66c does not correlate with that of CEA.[19,20] CD66c is a cell adhesion molecule that mediates homotypic and heterotypic cell-cell interaction with other CEACAM family molecules through integrin receptors.[21,22]

Normal human epithelial and myeloid cells express CD66c but at levels 1 to 2 log lower than malignant tissue.[23] CD66c overexpression in malignant cells suggests it as a novel biomarker for the progression of malignancies, especially in pancreatic adenocarcinoma and a number of other cancers, including colorectal, gastric, biliary, breast, prostate, ovary, and lung carcinomas.[24–29] A comparison of CEA and CD66c expression in primary and metastatic cancer demonstrated that lung adenocarcinoma had higher CD66c levels than other histological types and had higher CD66c than CEA expression.[29] However, no studies have measured CD66c overexpression in lung adenocarcinoma-associated malignant pleural effusion (LA-MPE).

In the current study, we investigated the diagnostic performance of CD66c in differentiating LA-MPE from benign pleural effusion (BPE) and compared it with other tumour markers, CEA, CA 19-9, and CYFRA 21-1.

MATERIALS AND METHODS

Sample collection and processing

Ninety-nine pleural effusion samples were prospectively collected from February 2009 to January 2013 at the Department of Pathology of Chungbuk National University Hospital (Cheongju, Korea). They were obtained from 47 patients with LA-MPEs and 52 with BPEs, including tuberculosis (24 cases), paraneumonic effusion (22 cases), and transudate from congestive heart failure, liver cirrhosis, and rheumatoid disease (6 cases). All cytology slides were screened by two cytotechnicians and two pathologists independently. Each

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LA-MPE was confirmed cytologically by the presence of adenocarcinoma cells. Cell blocks were prepared for 40 of the 47 LA-MPEs. The cells in these blocks were confirmed as adenocarcinoma by immunocytochemical staining for thyroid transcription factor-1 (SPT24, 1:200; Novocastra, UK) and cytokeratin 7 (OV-TL-12/30, 1:400; Novocastra) (Supplementary Table 1, http://links.lww.com/PAT/A29). The tumour cell percentage within each MPE cell block was determined independently by two pathologists, and the average value taken (Supplementary Table 1, http://links.lww.com/PAT/A29). Patients with other primary malignancies or with histological types other than adenocarcinoma were excluded. Tuberculosis was diagnosed by culture of pleural effusion, histopathological examination of a bronchial biopsy, or by polymerase chain reaction. Parapneumonic pleural effusion indicates non-empyemic effusion accompanying pneumonia. Other transudate BPEs were collected from patients with documented heart failure, liver cirrhosis, or rheumatoid disease, but without neoplastic disease. The supernatants of pleural effusions were obtained by centrifugation, and aliquots were stored at −80 °C. This study was conducted under approval of the institutional review board of the Chungbuk National University Hospital, Cheongju, Korea.

Immunoassays for CD66c, CEA, CA 19-9, and CYFRA 21-1

CEA and CA 19-9 in pleural effusions were detected by an electrochemiluminescence immunoassay (Roche Cobas 8000 modular analyser series; Roche Diagnostics, USA). CYFRA 21-1 concentrations were measured by a chemiluminescent enzyme immunoassay (Lumipulse G1200; Fujirebio, Japan). The CD66c immunoassay was performed with an enzyme-linked immunosorbent assay (ELISA) kit (DuoSet ELISA Development Kit; R&D Systems, USA) according to the manufacturer's instructions.

Immunocytochemical staining for CD66c, CEA, and CA 19-9

Immunocytochemical staining of CD66c, CEA, and CA 19-9 proteins was carried out in ethanol fixed, paraffin embedded cell blocks of pleural effusions obtained from 40 of 47 LA-MPEs and 20 of 52 BPEs. Immunocytochemistry was performed on 4 μM paraffin sections of cell blocks. Fully automated immunostaining was performed on a Benchmark XT autostainer (Ventana Medical Systems, USA).

Epitope retrieval was performed using CC1 solution (Tris-EDTA buffer, pH 8.4; Ventana) for CD66c, CEA, and CA 19-9. The slides were treated with 3% hydrogen peroxide for 4 min at 37°C to block endogenous peroxidase activity and rinsed between steps with Ventana Tris-based Reaction buffer. The slides were incubated with primary antibodies against CD66c (9A6, 1:3000; Santa Cruz, USA) for 1 h, CEA (12-140-10, 1:160; Novocastra) for 40 min, and CA19-9 (121SLE, prediluted; Ventana) for 16 min followed by Ventana Universal HRP Multimer (8 min at 37°C). Diaminobenzidine (DAB) was used as a chromogen and haematoxylin as a nuclear stain. Immunoreactions were detected using the using the Ultraview Universal DAB detection kit (Ventana). The reactions were visualised with 3,3'-diaminobenzidine, followed by counterstaining with haematoxylin. Appropriate positive and negative controls were used for each antibody. The distribution of staining was scored as negative or positive. Cytoplasmic staining of >5% of tumour cells was considered positive for all markers.

Statistical analysis

Continuous data were expressed as medians and minimum-maximum ranges. Differences between two independent groups were determined by the Mann–Whitney U test. The receiver operating characteristic (ROC) curves were calculated by logistic regression with the malignant/non-malignant condition as a dependent variable and the various tumour markers as independent variables. The areas under the curve (AUC) were calculated to determine the diagnostic value of each marker in pleural fluid. Results from patients with MPEs were used to select cut-off values for sensitivities and specificities for all markers. Analysis of the correlation between the result of enzyme immunoassay and immunocytochemistry was performed using the Mann–Whitney U test and Fisher's exact test. p values <0.05 (two-sided) were considered statistically significant. SPSS 12.0 software (IBM, USA) was used for statistical analysis.

RESULTS

Patient characteristics

Of the 99 pleural effusions, 47 (47.5%) were LA-MPEs and 52 (52.5%) were BPEs. Of the 47 patients with LA-MPEs, 24 were men (51.1%) and 23 were women (48.9%), with a mean age of 68 years (range 33–92). Of the 52 patients with BPEs, 39 were men (75.0%) and 13 were women (25.0%), with a median age of 65 years (range 22–92) (Table 1).

Diagnostic performance of single tumour markers by enzyme immunoassay

The median levels of CD66c, CEA, CA 19-9, and CYFRA 21-1 are shown in Table 2. The levels of all tumour markers were significantly higher in LA-MPEs than in BPEs (Mann–Whitney U test, $p < 0.001$). The sensitivity, specificity, and AUC values of the four tumour markers are shown in Table 3. The ROC curves of CD66c, CEA, CA 19-9, and CYFRA 21-1 for distinguishing LA-MPE from BPE are shown in Fig. 1. Cut-off points for each marker were determined by the maximum sum of sensitivity and specificity. Cut-off points were 5.8 mg/L, 2.9 mg/L, 22.6 mg/L, and 34.3 mg/L for pleural effusion concentrations of CD66c, CEA, CA 19-9, and CYFRA 21-1, respectively. CD66c was the tumour marker of the highest specificity (98.1%), with the sensitivity of 63.8%, and the AUC value of 0.815 (95% CI 0.722–0.909). CEA showed the best diagnostic values, with a sensitivity of 87.2%, specificity of 92.3%, and AUC of 0.914 (95% CI 0.848–0.981). The sensitivity and specificity were 55.3% and 98.1% by CA 19-9, and 83.0% and 76.9% by CYFRA 21-1, respectively. The AUC value of CA 19-9 and CYFRA 21-1 were 0.783 (95% CI 0.688–0.877) and 0.832 (95% CI 0.752–0.912), respectively.

Diagnostic performance of tumour marker combinations by enzyme immunoassay

Of the combination of two markers, the CEA and CA 19-9 pair had the highest sensitivity of 91.5% and a AUC of 0.933 (95% CI 0.869–0.996), with a specificity of 98.1%. The CEA and CYFRA 21-1 pair had the next highest AUC value of 0.930 (95% CI 0.882–0.979), with a sensitivity of 89.4%, and specificity of 86.5%. Of the combination of three markers, the CD66c/CEA/CYFRA 21-1 combination had the second highest values, with a sensitivity of 89.4%, specificity of 86.5%, and AUC value of 0.933 (95% CI 0.886–0.980). The CEA/CA 19-9/CYFRA 21-1 combination was the best diagnostic, with a sensitivity of 91.5%, specificity of 86.5%, and AUC value of 0.943 (95% CI 0.897–0.988). The combination of all four tumour markers increased the AUC value to 0.946 (95% CI 0.901–0.990) (Table 3).

Table 1 Patient characteristics

	No. patients (%)	
	BPE ($n = 52$)	LA-MPE ($n = 47$)
Age		
Median	65	68
Range	22–92	33–92
Sex		
Male	39 (75.0)	24 (51.1)
Female	13 (25.0)	23 (48.9)
Diagnosis		
Tuberculosis	24 (46.2)	
Pneumonia	22 (42.3)	
Transudate	6 (11.5)	

BPE, benign pleural effusion; LA-MPE, lung adenocarcinoma-associated malignant pleural effusion.