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Diagnostic value of cancer antigen 15-3 (CA15-3) detected by monoclonal antibodies (115D8 and DF3) in exudative pleural effusions

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ABSTRACT: Pleural fluid levels of the cancer antigen 15-3 (CA15-3) detected by monoclonal antibodies (115D8 and DF3) were determined in 40 patients with carcinomatous pleural effusions and in 41 patients with tuberculous pleural effusions. Using a cut off level of 16 U/ml, 15 of the 40 carcinomatous fluids but none of the 41 tuberculous fluids were positive. Pleural fluid levels of CA15-3 were not correlated with those of carcinoembryonic antigen (CEA) or carbohydrate antigen 19-9 (CA19-9). Combined assay of CEA and CA15-3, or CA19-9 and CA15-3, increased the positive rate from 79 to 82% and from 67 to 73%, respectively. Measurement of pleural fluid CA15-3 levels are less useful in separating carcinomatous from tuberculous effusions than is measurement of CEA or CA19-9.

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Several monoclonal antibodies have been developed against human mammary carcinomas. Immunogens used were breast tumour cell lines, milk fat globule membrane and membrane enriched extracts of breast carcinoma metastases. CA15-3 is composed of two different antigenic determinants recognized by murine monoclonal antibodies (115D8 and DF3) that react with human milk-fat globule membranes [1] and membrane-enriched fraction of a human breast carcinoma [2].

In our clinical experience, common aetiologies of exudative pleural effusions are carcinoma and tuberculosis. We have already reported that CEA, CA19-9 and adenosine deaminase (ADA) are useful markers for differentiation between carcinomatous and tuberculous pleural effusions [3]. However, little has been reported on the pleural level of CA15-3. To assist in distinguishing carcinomatous and tuberculous effusions among the exudative pleural effusions, we measured CA15-3 levels in pleural fluid.

Materials and methods

Pleural fluids obtained from 40 patients with carcinomatous pleural effusions and 41 patients with tuberculous pleural effusions, confirmed by pleural biopsy or bacteriologic or cytologic study of the effusions, were frozen at −20°C and stored until tested. There were 18 men and 22 women, 39–88 yrs of age among the patients with carcinomatous pleural effusions and 32 men and 9 women, 17–86 yrs of age with tuberculous pleurisy. The carcinomatous pleural effusions included 38 primary lung carcinomas (31 adenocarcinomas, four epidermoid carcinomas, two small cell carcinomas and one large cell carcinoma) and two metastatic carcinomas (stomach and colon).

CA15-3 was measured by radioimmunoassay (RIA) (Centocor, Malvern, Pa, U.S.A.). The assay is a solid-phase forward sandwich test [4]. Monoclonal antibody 115D8 is immobilized on polystyrene beads and serves as the capture antibody. Monoclonal antibody DF3 is labelled with ^{125}I and used as a tracer. The assay has two 1 h incubations at room temperature with no pre-assay sample treatment. In the first incubation the antigen in the specimen is bound to monoclonal antibody 115D8 on the bead. In the second incubation ^{125}I-DF3 forms a complex with 115D8-antigen on the bead. The bound radioactivity is proportional to the antigen in the sample. Assay standards are prepared from breast carcinoma cell line ZR-75-1 and expressed as arbitrary units per ml.

CA19-9 was determined by radioimmunoassay forward sandwich method (Centocor) in 49 patients with carcinomatous pleurisy and 42 patients with tuberculous pleurisy. CEA was measured by RIA sandwich method (Dainabot) in 60 patients with carcinomatous pleurisy and 50 patients with tuberculous pleurisy.

Groups were compared by the Mann-Whitney U-test or chi-square test.

Results

CA15-3 concentrations in pleural fluid from 40 patients with carcinomatous pleurisy and 41 patients with tuberculous pleurisy were 3.0–1,900 U/ml, and 5.3–16 U/ml, respectively (fig. 1). No significant difference was observed between them. CA15-3 values in tuberculous pleural effusions did not show a normal distribution, and the highest level of CA15-3 in tuberculous pleural fluids was 16 U/ml. A CA15-3 level in pleural fluid above 16 U/ml was considered specific for malignancy in our series. When the cut-off value of 16 U/ml was taken to differentiate between carcinomatous (> 16 U/ml) and tuberculous effusions (≤ 16 U/ml), the diagnostic sensitivity of the CA15-3 assay for malignancy was 38%, the specificity 100%, the predictive value of positive results 100%, the predictive value of negative results 62% and the efficacy of the assay 69% (table 1). There was a significant differenee between two relative frequencies in carcinomatous and tuberculous pleurisy ($p < 0.001$).

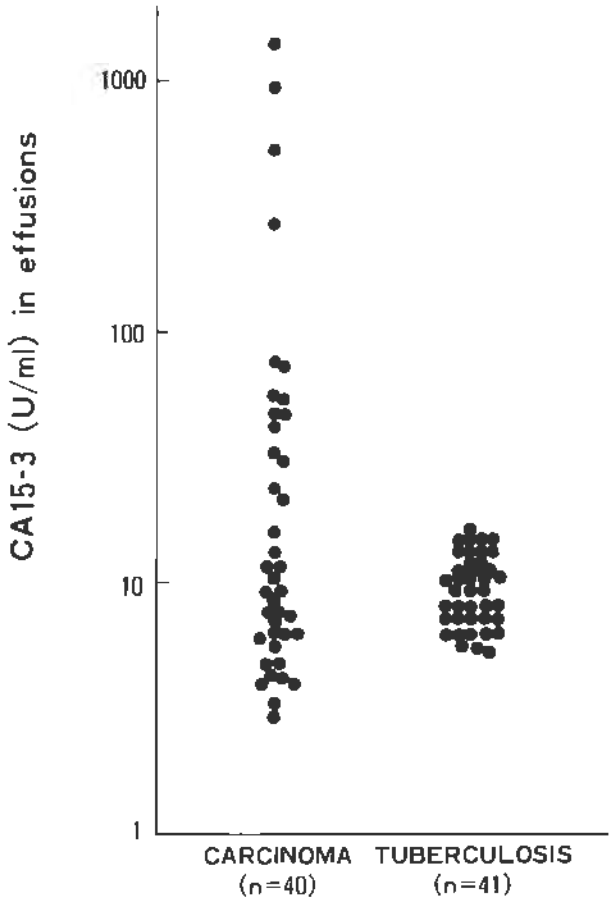

Fig. 1. CA15-3 levels in carcinomatous and tuberculous pleural effusions.

CA19-9 levels in carcinomatous and tuberculous effusions were 3–10,000 U/ml (n = 49) and 3–19 U/ml (n = 42). When 10 U/ml was taken as the cut-off, the diagnostic sensitivity of the CA19-9 assay for carcinomatous pleurisy was 61%, the specificity 95%, the predictive value of positive results 94%, the predictive value of negative results 68% and efficacy of the assay 77% (table 1). The difference between two relative frequencies in carcinomatous and tuberculous pleurisy was significant ($p < 0.001$).

CEA levels in carcinomatous and tuberculous effusions were 0.6–850 ng/ml (n = 60) and 0.5–3.9 ng/ml (n = 50). When 5 ng/ml was taken as the cut-off, the diagnostic sensitivity of the CEA assay for carcinomatous pleurisy was 77%, the specificity 100%, the predictive value of positive results 100%, the predictive value of negative results 78% and the efficacy of the assay 87% (table 1). The difference between two relative frequencies in carcinomatous and tuberculous pleurisy was significant ($p < 0.001$).

There were no correlations between CA19-9, CEA and CA15-3 values in carcinomatous effusions (figs 2 and 3). Therefore, CA15-3 was considered to be an independent marker from CEA and CA19-9. The positive rate in combined assay of CEA and CA15-3 increased from 79 to 82% when compared to CEA assay alone, and that in combined assay of CA19-9 and CA15-3 also increased from 67 to 73%, when compared to CA19-9 assay alone.

Table 1. - Accuracy of CA15-3, CA19-9 and CEA quantitation for diagnosis of malignant pleural effusions

	CA15-3[a]		CA19-9[a]		CEA[a]	
	n	%	n	%	n	%
Sensitivity[b]	15/40	38	30/49	61	46/60	77
Specificity	41/41	100	40/42	95	50/50	100
Predictive value of a positive test	15/15	100	30/32	94	46/46	100
Predictive value of a negative test	41/66	62	40/59	68	50/64	78
Efficacy	56/81	69	70/91	77	96/110	87

a) CA15-3 values above 16 U/ml, CA19-9 values above 10 U/ml and CEA values above 5 ng/ml were considered positive. b) Sensitivity: frequency of positive results in malignant effusions; specificity: frequency of negative results in nonmalignant effusions; predictive value of a positive test: frequency of malignant effusions in all patients with positive results; predictive value of a negative test: frequency of nonmalignant effusions in all patients with negative results; efficacy: percentage of effusions correctly classified (malignant and nonmalignant) by the test.

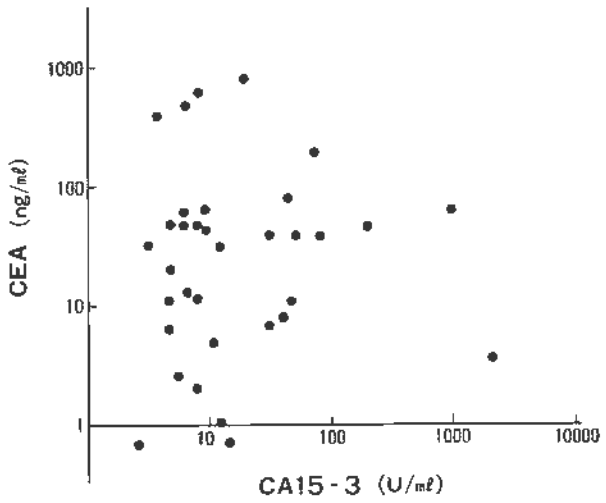

Fig. 2. Correlation between CEA and CA15-3 values in 34 carcinomatous pleural effusions.

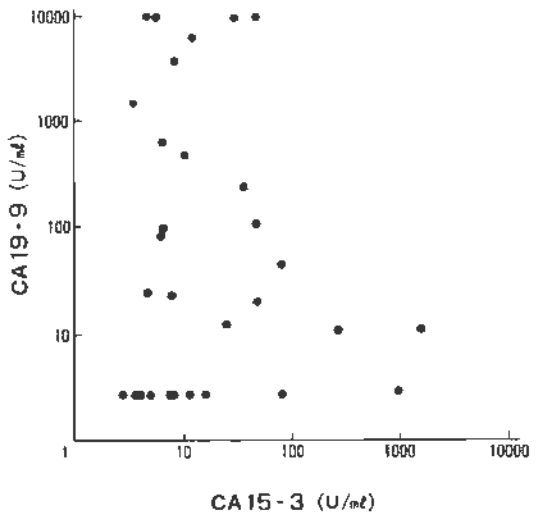

Fig. 3. Correlation between CA 19-9 and CA15-3 values in 30 carcinomatous pleural effusions.

Discussion

Using a radioimmunoassay for the detection of a monoclonal antibody defined breast tumour associated antigen 115D8/DF3, the mean serum value of 1051 healthy controls was 12.9 (SD 5.9) U/ml with 99% of the values below 29.5 U/ml [4]. With a double-determinant enzyme-linked immunoassay (EIA), only 6 of 111 normal subjects had EIA DF3 antigen level ≥ 30 U/ml [5].

There have been some reports on the serum levels of 115D8/DF3 antigen in patients with lung cancer, using a monoclonal antibody, 115D8 or DF3. No increased 115D8 antigen levels were found in sera from patients with lung cancer [1]. Only 1 of 11 (9%) patients with lung cancer had elevated circulating DF3 antigen levels [5]. Using two monoclonal antibodies 115D8 and DF3, 8 of 25 (32%) patients with lung cancer showed positive results in serum [6]. As mentioned above, serum levels of 115D8/DF3 antigen were low in patients with lung cancer. However, immune peroxidase staining of sections of formalin-fixed and embedded tumour sections by DF3 monoclonal antibody was positive in 32 out of 38 patients with lung cancer [7]. The reason for discrepancy between low circulating levels of DF3 antigen and high incidence of staining of lung cancer sections by DF3 monoclonal antibody is unclear. DF3 antigen might be more tightly bound to the cell membrane of lung cancer or might be secreted to a lesser degree.

However, no normal range of CA15-3 has been determined in pleural effusions. We set the cut-off level at 16 U/ml in pleural effusions. Then, the specificity was 100% and the sensitivity was 38%. Specificity and sensitivity of CEA in carcinomatous fluids were 100% and 77%. Those of CA19-9 were 95% and 61%. Comparing these figures, assay of CA15-3 alone in pleural fluid appears to be a relatively poor screening test for differentiating carcinomatous from tuberculous effusions although its specificity is high. In our series pleural effusions due to breast cancer were not involved. When these effusions are included in the study, the sensitivity might be expected to reach a higher level. We found no correlation between fluid levels of CA15-3, CEA and CA19-9 in patients with carcinomatous pleurisy. In fact, combined assay of CA15-3, CEA and CA19-9 in pleural fluid increased the incidence rate more than the measurement of CEA or CA19-9 alone.

Reactivity of monoclonal antibodies including DF3 has been studied with cells in human malignant and benign effusions [8]. Such a study may have research applications and therefore merits further investigation, although we did not attempt immunohistochemical staining of exudative pleural cells.

In conclusion, combined assays of CA15-3, CEA and CA19-9 in pleural effusions proved superior to determination of CEA or CA19-9 alone in discriminating between carcinomatous and tuberculous pleurisy.

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RÉSUMÉ: Chez 40 patients atteints d'épanchements pleuraux carcinomateux, et chez 41 patients à épanchements pleuraux tuberculeux, les niveaux de l'antigène cancéreux 15-3 (CA15-3) ont été déterminés dans le liquide pleural par des anticorps monoclonaux 115D8 et DF3. Chez 15 des 40 sujets porteurs d'épanchements carcinomateux, le taux de 16 unités/ml a été atteint, alors qu'il ne l'était dans aucun des 41 épanchements tuberculeux. Il n'y avait pas de corrélation entre les niveaux du CA15-3 dans le liquide pleural et ceux de l'antigène carcino-embryonnaire (CEA) ou de l'antigène carbohydrate 19-9 (CA19-9). L'essai combiné du CEA et du CA15-3, ou de CA19-9 et du CA15-3, a augmenté le taux de positivité respectivement de 79 à 82% et de 67 à 73%. La détermination des niveaux de CA15-3 dans le liquide pleural est utile pour la différenciation entre épanchement tuberculeux et carcinomateux, moins toutefois que les mesures du CA19-19 ou du CEA.