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Detection of Malignant Pleural Effusions by Tumor Marker Evaluation

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Abstract—*Cytologic examination and determination of tumor markers (PHI, LDH, alpha-1-glycoprotein, alpha-2-HS-glycoprotein, β2-microglobulin, feritin, sialic acid IgE, fetoprotein, CEA, βHCG and β1-SP-glycoprotein) were carried out in pleural fluid samples obtained from 70 patients with suspected neoplasia. Tumor markers were also determined in sera. The protein content of all pleural effusions was ⩾ 3 g/dl.*

Patients were grouped according to diagnosis as follows: (a) 42 withneoplastic diseases (7 mesotheliomas and 19 lung, 4 ovarian, 3 breast and 8 miscellaneous cancers), (b) 22 with benign inflammations and (c) 6 with congestive effusions.

Of the parameters examined, only CEA and α-HCG gave information that the effusionwas probably malignant. Using 6 ng/ml as cut-off for CEA and 10 mIU/ml for βHCG, the sensitivity was 57.1% and 45.2%, respectively, specificity was 92.8% for both parameters and test efficiency 0.75 and 0.69, respectively. When CEA and βHCG were considered together sensitivity increased to 73.8% and efficiency to 0.78. CEA and/or βHCG were positive in the pleural effusions of 19 of the 20 malignant pleural effusions, all with a negative cytologic examination,, which subsequently became positive in 8. Because of their high specificity, these two parameters are a useful tool and can be routinely measured to evaluate pleural effusions of dubious origin, even if CEA and βHCG cannot, in their own, define the primary malignancy.

INTRODUCTION

IN CLINICAL practice the etiology of pleural effusions is sometimes obscure. Often the origin is a malignancy and differentiation between malignant and non-malignant effusions takes priority. The cytologic examinations of pleural effusion is highly specific for the detection of malignant tumors, but false negative rates of 30–50% have been reported [1–3].

The diagnosis of the underlying pathology of malignant effusions is often difficult and biochemical analysis of the pleural fluid has been proposed for this purpose. Several parameters have been evaluated for differentiating malignant from benign inflammatory and congestive effusions. Some of these have been found to be of diagnostic significance, such as carcinoembryonic antigen (CEA), alpha-fetoprotein (AF), human chorionic gonadotropin (HCG), carbohydrate antigen 19-9 (CA 19-9), tissue polypeptide antigen (TPA), CA 125, β-2-microglobulin (β2μ), ferritin, alpha-1-glycoprotein (AAG) and the enzymes phosphohexose isomerase (PHI), lactate dehydrogenase (LDH) and adenosine deaminase [2–13]. The literature differs both in the data of the cut-off values of the markers adopted and for the contradictory conclusions reached concerning the discriminating value of these parameters [6, 10, 11, 14, 15].

The aim of this study was to determine the diagnostic value of biochemical analysis in a group of pleural effusions of unknown origin where malignancy was suspected. Some parameters among the above mentioned were investigated. In addition total sialic acid (NANA), alpha-2-HS-glycoprotein (a2HS), IgE and β1-SP-glycoprotein (β1SP) were also measured in pleural effusions and in corresponding sera and evaluated for their role as tumor markers [16–20].

The results were considered in light of the definitive diagnosis confirmed by pathologic examination.

MATERIALS AND METHODS

Sera and pleural fluids of 70 hospitalized patients with pleural effusion of unknown origin suspected secondary to malignancy were investigated.

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On the same day, both blood and pleural fluid were collected from each patient. A sample of pleural fluid was submitted for routine cytologic examination. Serum and supernatant of the fluid obtained by centrifugation at 3000 rpm for 15 min were divided among several tubes and stored at −20°C until examined (no more than 2 months, depending on the investigated parameter). Diagnoses were established by clinical laboratory and RX evaluation and in all the considered patients, confirmed by pathology.

In both pleural effusions and sera the concentration of the following were determined without any knowledge of the definitive diagnosis: total proteins, albumin, LDH, PHI, AAG, a2HS, NANA, ferritin, β2μ, IgE, CEA, AF, βHCG and β1SP.

Total protein concentration was determined by the biuret method using a centrifugal analyzer; serum reference values (s.r.v.) were 6–8 g/dl. Albumin concentration was measured by densitometry following cellulose acetate electrophoresis (s.r.v.: 3.2–6.2 g/dl). PHI and LDH activities were determined by optimized u.v. methods (Behring, Marburg, F.R.G. and Biochemia, Mannheim, F.R.G., respectively) using a centrifugal analyzer (s.r.v.: 15–75 U/l and <240 U/l, respectively). AAG was evaluated by an immunonephelometric method using an ICS Beckman analyzer (s.r.v.: <100 mg/dl), and a2HS by radial immunodiffusion (Behring) (s.r.v. for a population of 100 healthy people: 44–64 mg/dl). NANA was measured by a colorimetric enzymatic method (Biochemia) adapted to a COBAS-BIO (Roche, Basel, Switzerland) centrifugal analyzer (s.r.v.: <70 mg/dl).

Immunoenzymatic techniques were used to measure the other parameters. Ferritin and AF concentrations were determined using the Biochemia kit and on a ES 22 analyzer; ferritin concentrations > 400 ng/ml were considered pathological, AF s.r.v. was < 12 IU/ml. CEA (s.r.v.: < 5 ng/ml and βHCG (s.r.v.: < 12 mIU/ml) were measured using ROCHE EIA kits, while Behring kits were used for IgE, β2μ and β1SP with the following s.r.v.: < 100 IU/ml, < 2.5 mg/l and < 1 μg/l, respectively. For immunoenzymatic methods, the samples which fell out of the linear range of the calibration curve (very often more restricted than that declared by the reagent manufacturer), were appropriately diluted using the correspective zero standard.

At least two cut-off values were considered for each parameter of pleural fluid by evaluating three types of concentrations: (1) as an absolute, (2) per g of total proteins and (3) per g of albumin. For each cut-off defined, sensitivity (percentage of the malignancies with parameter concentration above the cut-off), specificity (percentage of the non-malignant sera or fluids with parameter concentration below the cut-off) and test efficiency (true positives plus true negatives divided by the total examined) were calculated.

RESULTS

The 70 patients were grouped according to diagnosis as follows:

Group A: 42 with neoplasia; 19 lung cancers, 7 mesotheliomas, 4 ovarian cancers, 3 breast cancers and 8 miscellaneous cancers. The presence of neoplasia was determined by cytology, biopsy or *post mortem* examination.

Group B: 22 with bacterial infections.

Group C: 6 with congestive effusions due to liver damage or heart failure.

In 22 of the 42 neoplastic patients (52.38%) the search for malignant cells in pleural fluid was positive at the first examination. In 8 of the remaining 20, cytologic examination was positive at the second or successive attempts. In conclusion, the false negative rate for cytologic examination was 28.6%. All samples from groups B and C were negative.

Total serum protein and albumin concentrations were normal in all 70 patients. In all pleural fluids total proteins were ≥ 3.3 g/dl and albumin ≥ 1.8 g/dl.

The best discrimination between the three groups of patients was obtained considering the concentrations of the biochemical parameters as absolutes. No serum or pleural fluid revealed the presence of AF. The other parameters showed a wide range of concentrations and, in most cases, these were higher in pleural fluids than in the corresponding sera.

Table 1 lists *r* values between serum and pleural fluid concentrations. AAG and IgE correlate in all the three groups, a2HS in groups A and B, while several parameters correlated only in group C.

Figures 1–4 show the results in pleural fluids. β2μ and a2HS were unable to differentiate any group. PHI and LDH were similarly distributed in groups A and B with lower concentrations in C. Ferritin, AAG, NANA and β1SP showed a similar behavior, but not so close as that of the enzymes (in one case in group C, with heart failure, high concentrations of the four parameters were observed). CEA and βHCG were very valid in differentiating between the 3 groups.

Table 2 reports sensitivity, specificity and test efficiency of 11 investigated parameters at different cut-offs and of the association CEA + βHCG. A specificity round about 90% was considered. Too often this level of specificity gives very low sensitivity. The best test efficiency is given by CEA and βHCG and an improvement is obtained when the two parameters are considered together.

Table 1. r *values between serum and the correspective pleural fluid concentration of the evaluated markers in the three groups of patients*

	r serum – pleural fluid					
	Group A		Group B		Group C	
	n	*r*	*n*	*r*	*n*	*r*
LDH	42	0.24	21	0.09	6	0.62
PHI	42	0.16	21	0.01	6	0.69
AAG	41	0.86	22	0.92	6	0.88
a2HS	33	0.81	16	0.77	6	0.28
Sialic acid	40	0.38	22	0.75	6	0.95
Ferritin	41	0.27	21	0.11	6	0.17
β2μ	41	0.50	22	0.64	6	0.73
IgE	36	0.98	19	0.96	5	0.99
CEA	42	0.007	22	0.53	6	0.22
β-HCG	42	0.25	22	0.31	6	0.87
β1-SP	42	0.19	21	0.53	6	0.97
Total protein	40	0.49	22	0.61	6	0.42
Albumin	40	0.46	22	0.58	6	0.71

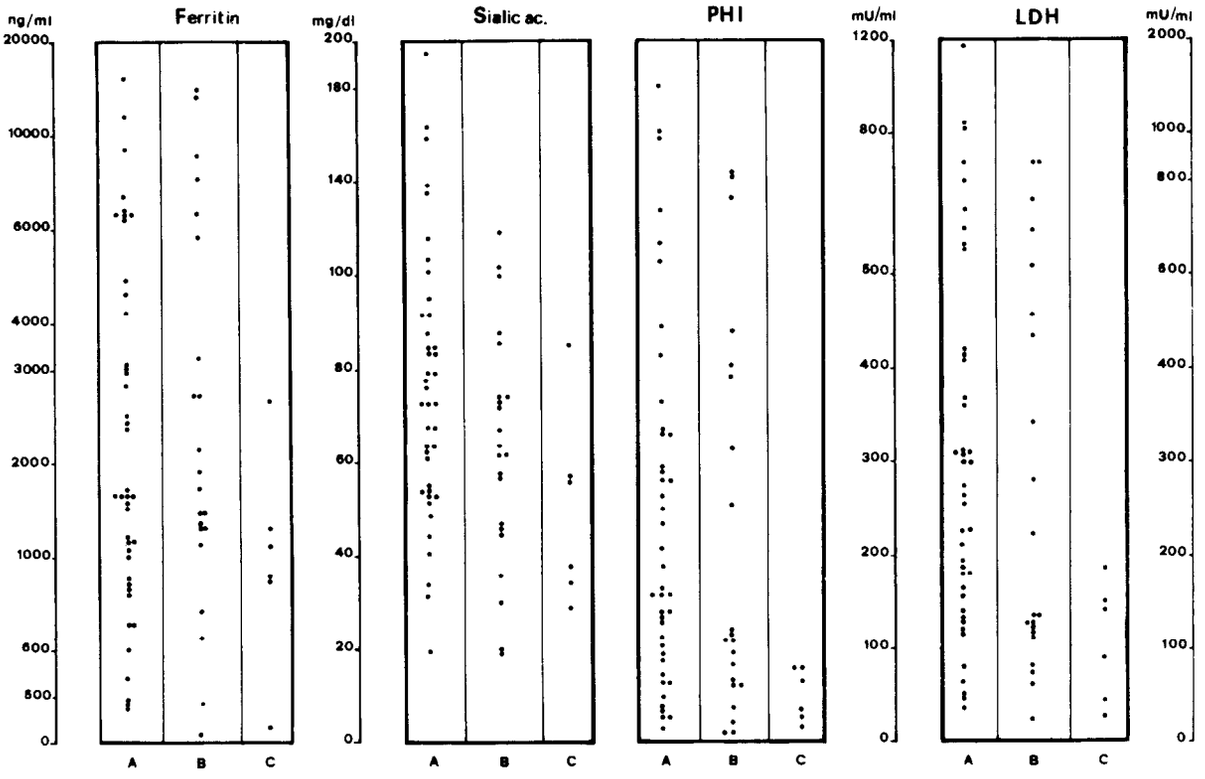

Fig. 1. *Concentration values of ferritin, sialic acid, PHI and LDH in the examined pleural fluids. A: malignant, B: benign inflammations, C: congestive.*

CEA ⩾ 6 ng/ml and/or βHCG ⩾ 10 mIU/ml were found in 19 of 20 pleural fluids which gave negative results at the first cytologic examination and in all (28.57%) which were false negative CEA + βHCG + cytology gave 100% sensitivity. CEA + βHCG were unable to detect (absence of sensitivity) the presence of malignancy in 11 malignant effusions (26.2% of total): 5 mesotheliomas, 1 thymoma, 2 ovarian and 3 lung cancers.

DISCUSSION

Comparing serum and pleural fluid concentrations, *r* values and serum and pleural fluid albumin it appears that, among the parameters examined, PhI, LDH, ferritin, β2μ, CEA and βHCG are locally produced or selectively accumulated in pleural fluids. The diagnostic value of these parameters determined in pleural fluids does not improve by their evaluation in the corresponding

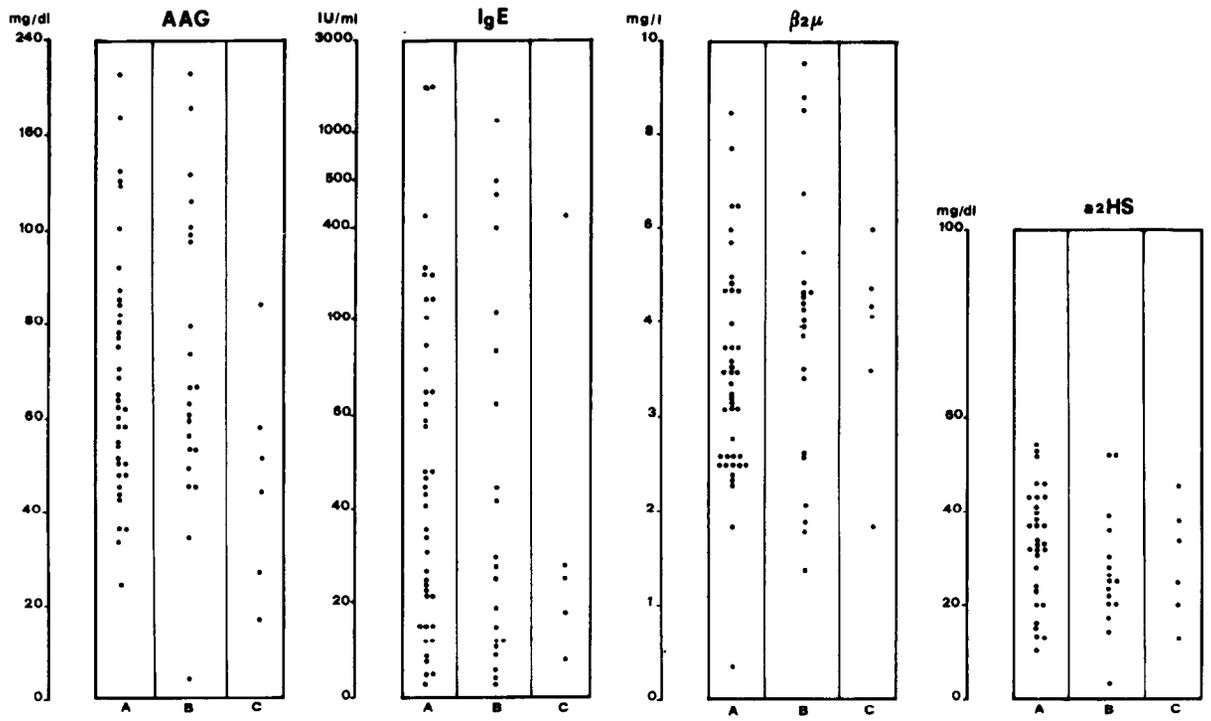

Fig. 2. Concentration values of AAG, IgE, β2μ and a2HS in the examined pleural fluids. A: malignant, B: benign inflammations, C: congestive.

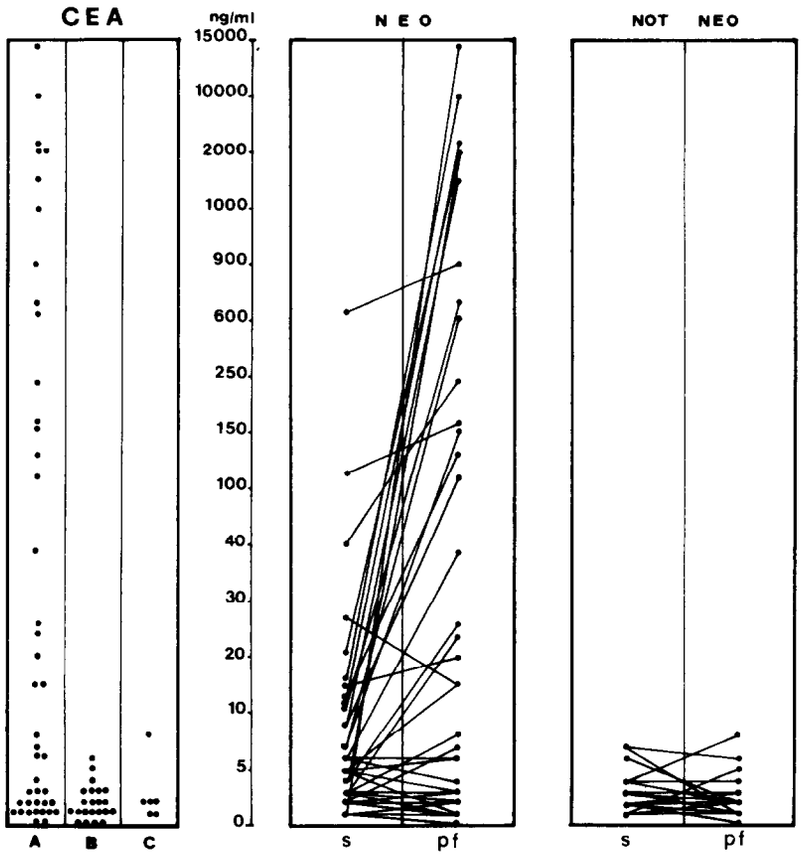

Fig. 3. CEA concentrations in serum and pleural effusions. A, B and C: malignant, due to benign inflammations and congestive pleural fluids, respectively. s: serum, pf: pleural fluid.

serum. On the other hand, IgE, AAG, a2HS and sialic acid seem to derive from serum. The enzymes LDH and PHI paralleled each other and were confirmed [3, 11] to discriminate congestive effusions from inflammatory benign and malignant ones, but not between these latter two. The high

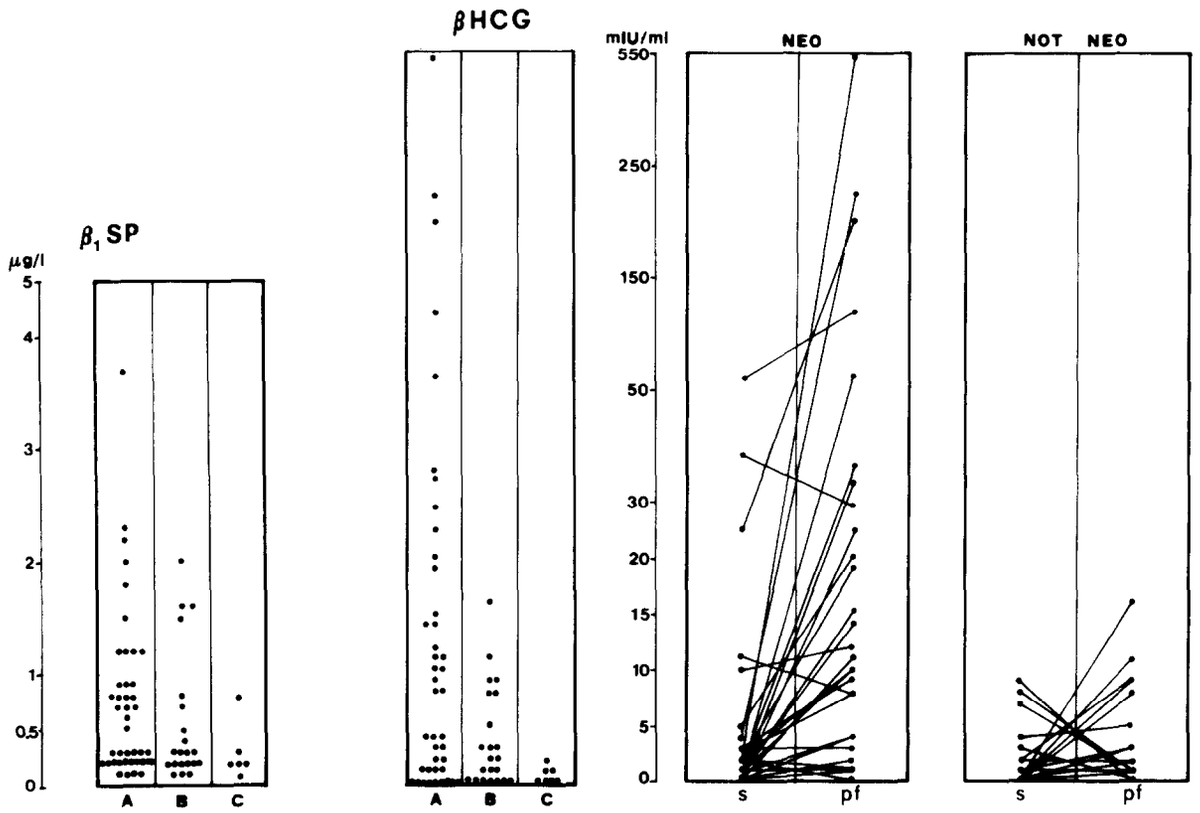

Fig. 4. Concentrations of β1SP in the pleural fluids and of βHCG in serum and pleural fluids. A: malignant, B: benign inflammations, C: congestive. s: serum, pf: pleural fluid.

enzyme activities should therefore be considered to derive from an aspecific inflammatory reaction to processes of various origin and not from production by neoplastic cells. Low levels of activities did not exclude the presence of malignant effusions, which is in contrast to that observed by Martinez-Vea *et al.* for PHI [3].

Alpha-2-HS was of no use in pleural fluid evaluation: metabolism of this protein is very complex [16, 21] and further studies are necessary to assess its role in the presence of malignancy.

Mean AAG and sialic acid concentrations decreased from group A to B to C, however the wide overlap between the three groups made the test efficiency very poor. Our findings concerning AAG confirm those observed by others [9, 11] and contrast other studies which propose AAG as a reliable tumor marker [3, 5], in particular we found that low concentrations of AAG do not exclude malignancy as suggested by Martinez-Vea *et al.* [3].

The highest ferritin levels for malignant pleural effusions have been found in mesotheliomas, with a marked overlap with bronchogenic carcinoma [8]. Our results do not confirm this finding: ferritin levels over 6000 ng/ml were observed in two cases of mesothelioma, two lung, one prostate, one thyroid and one breast cancer, one sarcoma and one neoplasm of unknown origin. Ferritin did not discriminate between malignant, inflammatory and congestive effusions. This is in agreement with the observation that pleural fluid ferritin concentration comes from both pleural histiocytes and inflammatory cells (lymphocytes and granulocytes) and not from malignant cells [7, 8].

The wide overlap of β2µ values in the three groups of patients makes this protein of no diagnostic value. The same is also true for IgE. This immunoglobulin was significantly lower in group C than in the others, however one case of pleural effusion due to heart failure revealed repeatedly high values. Moreover, the close correlation between serum and pleural fluid IgE concentrations makes determination in effusions unnecessary.

Beta-1-SP, detected by immunohistological methods, has been reported to be helpful in distinguishing malignant mesothelioma, in which it is not found, from pulmonary adenocarcinoma [17]. Findings different from those expected were obtained in pleural fluids: the protein content was $\geq$ 1 µg/l in 10 cases: 3 mesotheliomas, 2 lung, 2 ovarian, 1 stomach and 1 breast cancer and the last a tumor of unknown origin. This low specificity may be due to a too high cut-off value (1 µg/l), however the test is imprecise and therefore loses validity below this value.

The other two markers we considered, CEA and

Table 2. Sensitivity, specificity and efficiency of the tumor markers examined in sera and pleural fluids according to the different cut-offs. n ex.: total examined in serum and pleural fluid, respectively; s.: serum; p.f.: pleural fluid

Parameter	Cut-off	Sensitivity %			Specificity %			Efficiency	
	< x	*n* ex.	s.	p.f.	*n* ex.	s.	p.f.	s.	p.f.
LDH	240 mU/ml	42	16.6	54.7	27	81.4	66.6	0.49	0.60
	480 mU/ml		4.7	21.4		100	77.7	0.52	0.49
PHI	75 mU/ml	42	69.0	78.5	27	33.3	40.7	0.51	0.59
	200 mU/ml		7.1	47.6		92.5	70.3	0.48	0.58
	400 mU/ml		0.0	19.0		100	81.4	0.50	0.50
AAG	80 mg/dl	41	80.4	31.7	28	21.4	71.4	0.50	0.51
	100 mg/dl		56.1	14.6		60.7	82.1	0.58	0.48
	140 mg/dl		17.0	4.8		75.0	92.8	0.46	0.48
a2HS*	>50 mg/dl	37–33	40.5	90.9	24–22	50.0	9.1	0.45	0.50
	>44 mg/dl		35.1	84.8		54.1	13.6	0.44	0.49
	>30 mg/dl		13.5	33.3		95.8	36.6	0.54	0.33
Sialic acid	70 mg/dl	40–42	92.5	54.8	28	7.1	64.2	0.49	0.59
	100 mg/dl		77.5	19.1		25.0	89.2	0.51	0.54
Ferritin	400 ng/ml	41–42	53.6	97.6	27–28	59.2	7.1	0.56	0.52
	4000 ng/ml		2.4	28.5		100	78.5	0.51	0.53
	10000 ng/ml		0.0	4.7		100	92.8	0.50	0.48
β2μ	2500 ng/l	41–42	56.0	88.1	28	46.4	17.8	0.51	0.52
	5000 ng/l		9.7	16.7		78.5	78.6	0.44	0.47
	6000 ng/l		7.3	11.9		92.8	82.1	0.50	0.47
IgE	50 IU/ml	36–41	69.4	39.1	24–26	50.0	69.2	0.59	0.54
	150 IU/ml		33.3	17.3		75.0	80.7	0.54	0.49
β1-SP	0.5 μg/l	42	39.9	52.3	27–28	70.3	71.4	0.55	0.61
	1 μg/l		16.6	23.8		88.8	85.7	0.52	0.54
	1.7 μg/l		4.7	11.9		96.2	96.4	0.50	0.54
CEA	5 ng/ml	42	50.0	57.1	28	89.2	89.2	0.69	0.73
	6 ng/ml		40.4	57.1		89.2	92.8	0.64	0.75
βHCG	5 mIU/ml	42	14.2	52.3	28	85.7	75.0	0.49	0.63
	10 mIU/ml		11.8	45.2		100	92.8	0.55	0.69
	12 mIU/ml		7.1	35.7		100	96.4	0.53	0.66
	17 mIU/ml		7.1	26.1		100	100	0.53	0.63
CEA βHCG	(6 ng/ml) + (10 mIU/ml)	42	57.1	73.8	28	89.2	85.7	0.74	0.78

*Only values of a2HS *below* the cut-off were considered pathological.

βHCG, appeared to be of help in assessing diagosis. Specificity and sensitivity were similar to those reported by other authors; the association of the two parameters improved test efficiency [3, 4, 9, 11, 14]. We think that the cut-off values of 6 ng/ml for CEA and 10 mIU/ml for βHCG (with a specificity of 92.8% in both and a sensitivity of 57.1 and 45.2%) show that the effusion is probably malignant.

In conclusion, we believe that CEA and βHCG are the only parameters, along with cytological investigation, which should be routinely determined in pleural fluids of unknown origin with a suspicion of malignancy. This also serves to control laboratory investigation costs. The presence of these markers is often diagnostic or contributes to hold the suspicion of malignancy when cytological investigation is negative, even if CEA and βHCG, on their own, cannot define the primary.

Even though CEA + βHCG + cytologic investigation gave 100% sensitivity in our patients, this finding cannot be considered as an absolute for the limited number of people investigated. In fact cytology, CEA and βHCG may all be negative in certain malignancies, such as mesotheliomas. Other tumor associated antigens, such as CA 19-9, CA 15-3, CA 50, etc., can then be investigated if the suspicion of a malignant effusion still exists, always considering that aspecific reactions are possible, as observed for CA-125 [22]. In the course (or suspicion) of a recurrence of a known malignant primary the investigative approach is quite different and the choice of tumor associated antigens to investigate is more specific.

The investigation of the other parameters (LDH, PHI, AAG, a2HS, IgE, ferritin, NANA, β2μ), is unnecessary in suspected malignant pleural effusions for their very poor diagnostic value.

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