Expresion of immunocytochemical markers on tumour cells and mesothelial cells in relation to cancer metastasis

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ABSTRACT

One of the main challenges of diagnostic cytology is finding an unequivocal and reliable method for discriminating between benign reactive mesothelial cells and malignant cells exfoliating into peritoneal effusions. Cytomorphologic examination alone provide only limited sensitivity for the detection of metastatic carcinoma cells in many cases of serous effusions. The ability to detect cancer by molecular analysis in body fluids depends mainly on the enrichment of tumor cells in the specimens. Early diagnosis and management of peritoneal metastases from cancer patients are difficult clinical challenges. The aim of this study was to assess the practical value of immunocytochemical techniques in routine cytological diagnosis, showing the cytopathological and immunocytochemical aspects encountered in 77 available cases with a histological diagnosis known. The cases were chosen to show typical cytological patterns (both benign and malignant) and in this way to achieve a diagnostic value of the immunocytochemistry method. Immunocytochemistry is routinely used in hospital laboratories to diagnose cancers, identify infectious organisms, differentiate malignant from benign processes, and reveal prognostic indicators. A panel of tumor markers (monoclonal antibodies), was applied to smears of cell deposit from peritoneal fluids, using avidin–biotin complex method, LSAB (HRP). It is concluded that a suitably chosen panel of monoclonal antibodies can be of great value in identifying neoplastic cells in serous effusions. Determination of effusions-specific immunostaining profiles is valuable in the diagnostic differentiation of metastatic carcinomas from common primary sites and should help to correctly predict the organ of primary tumor origin.

Key words: immunocytochemistry, cytopathology, peritoneal effusions, benign, malign

INTRODUCTION

Immunocytochemistry, a technique used for the localization of specific cellular antigens, was introduced to diagnostic tumor pathology about 20 yr ago. The procedure allows for the visualization of antigens in tissue samples via the sequential application of a specific antibody to the antigen (primary antibody), a secondary antibody to the primary antibody, an enzyme complex, and a chromogenic substrate. The enzymatic activation of the chromogen results in a

visible reaction product at the antigen site, which is interpreted using a light microscope.

Various studies have shown a sensitivity of 57.3% and specificity of 89% by conventional cytology for the detection of malignant cells in effusion samples (1, 2).

The management of malignant ascites of unknown primary tumor is in evolution. Repeated abdominal paracentesis may be useful in reducing symptoms. Intraoperative placement of Foley's catheter for continuous drainage of malignant ascites carries a high mortality rate (3). Intraperitoneal instillation of radioisotope chromic phosphate colloid, immunotherapeutic agents such as trifunctional antibodies, endostatin adenoviral vector and triamcinolone hexacetanide have been tried. Octreotide as an agent for palliative care has also been studied (4).

Malignant peritoneal effusions contribute to considerable morbidity in cancer patients and generally portend an overall poor prognosis. Treatment of malignant peritoneal effusions is palliative, therefore, quality of life issues, as well as the risks and benefits of the therapeutic options, become more critical. Malignant ascites are defined generally as ascites containing malignant cells; therefore, in most studies, efforts have been made to detect free cancer cells in peritoneal cytology specimens. In 10-20% of cases, the primary tumor may be difficult to detect and presents as a clinical enigma (5). Ascitic fluid analysis, tumor markers, radiological imaging and even diagnostic laparoscopy may fail to detect the primary tumor. Malignant cells will be detected in ascitic fluid in nearly 40-60% of patients (6). Due to the limited survival rate, supportive and symptomatic therapy remains the mainstay of the management and an aggressive approach is not indicated. It is difficult to compare results and determine the true efficacy of different techniques and agents because endpoints and response criteria as well as the extent and method of follow-up vary. Malignant peritoneal effusions harbor tumor cells, shed from direct extension or metastatic spread, and are due to impaired reabsorption or an increased production of peritoneal fluid. Effusions in cancer patients are not always "malignant" but may be reactive due to related to comorbid conditions. Nonetheless, effusions of malignant origin are usually recurrent, cause considerable morbidity in cancer patients, and are generally associated with a poor prognosis (7).

Cytopathology is no longer simply a screening modality limited to the "Pap mills" of yore.

Whether it is the detection of specific proteins/ antigens for definitive diagnoses/treatment guidance in immunotherapy, or it is "reading nucleic acids," the cytopathologist of the future will be called upon to gather and report more detailed and precise information. As we develop methods for extrapolating the secrets previously locked within the individual cells, it becomes evident that the cells were talking all along, we just did not know how to listen.

Pleura and peritoneum are common sites of metastatic tumor manifestation. The evaluation of serous effusions for metastatic carcinoma cells is critical for the therapy and for determining the prognosis of the disease. Owing to the scarcity of tumor cells in malignant effusions or morphologic similarities to reactive mesothelial cells, cytologic examination fails to detect malignant cells in approximately 40% of effusions (2). Therefore, in many cases, more invasive techniques such as biopsy, thoracoscopy or thoracotomy might be unnecessary to obtain a definitive diagnosis (8).

The rate of diagnostically equivocal effusions in routine cytology is dependent on the volume of effusion examined, type of preparation and staining, experience of the examiner, and application of ancillary methods. Peritoneal effusions are a frequently encountered clinical manifestation of metastatic disease, with breast, ovarian, and lung carcinomas and malignant mesothelioma leading the list (9).

The first report of an intraoperative examination of peritoneal cytology to detect subclinical metastases was presented in 1971. Patients with normal peritoneal cytological specimens had better survival rates than patients with abnormal findings, but only one abnormal cytologic specimen was found in early stage disease (10) Abdominal compartment syndrome and spontaneous bacterial peritonitis have been reported with untreated malignant ascites (11, 12). However, the median survival of such patients is usually measured in days.

The cytological diagnoses of serous effusions are usually made by routine cytomorphology with certainty, allowing treatment decisions. Various studies have shown a sensitivity of 57.3% and specificity of 89% by conventional cytology for the detection of malignant cells in effusion samples (2). Studies have shown that positive and negative predictive values for detection of malignancy by cytomorphology are 89.3% and 69.4% respectively. However, a grey zone always exists, where the cytopathologist encounters problems in determining the nature

of the cells whether reactive, atypical, or beyond doubt malignant (13). Therefore various ancillary techniques should be used to increase the diagnostic accuracy of malignancy in serous effusions. Primary cytomorphologic criteria of malignancy include cellular aggregates, pleomorphism (variable cellular appearance), anisocytosis (variation in cell size), anisokaryosis (variation in nuclear size), multinucleation, prominent to irregular nucleoli, increased nuclear to cytoplasmic ratio, monomorphic cellular appearance, and increased mitotic figures. Hyperplastic mesothelial cells also may exhibit anisocytosis, anisokaryosis, increased nuclear to cytoplasmic ratio, binucleate and multinucleate, and scattered mitoses. Any situation that results in fluid accumulation within the body cavities can induce mesothelial cell hyperplasia and exfoliation with an abnormal cellular morphology (14). Therefore, the differentiation between mesothelial cell hyperplasia and mesothelioma may be difficult or impossible.

MATERIALS AND METHODS

This was hospital based prospective study carried out in Emergency Clinical Contry Hospital of Constanta – Department of Pathology from September 2007 to May 2009. Clinical charts of all the patients whose peritoneal fluid samples were sent for cytological examination during the studied period, were retrieved for relevant information. Histopathologic examination of tissue was used as gold standard to diagnose malignancy, in cases where this was available. Follow-up data were obtained from the Tumor Registry of ECCHC.

The studied material consisted out of 77 body cavity fluid specimens (peritoneal effusions), produced in various malignant and benign conditions, from 45 (58,44%) women and 32 (41,55%) men. The majority of carcinomas were ovarian carcinomas (n = 18) with the remaining specimens histological diagnosed as gastrointestinal carcinoma (n = 9), pulmonary carcinoma (n = 4), breast carcinoma (n = 3) and peritoneal mesothelioma (n = 2). The histologic grade was excluded. Paracentesis was done, largely for symptomatic relief. If no fluid was present, the peritoneal cavity was lavaged with saline solution, and the fluid was then collected for analysis. Effusions were submitted for routine diagnostic purposes and the diagnoses were established by evaluation of smears. Confirmation of the origin of carcinoma was difficult to be made from the effusion cytology.

May-Grunwald-Giemsa and Papanicolaou stained slides were prepared from sediment obtained by centrifuging the peritoneal liquid samples at 1500 rpm for 5 minutes, using Shandon Cytospin preparations.The cytologic diagnosis was supported subsequently by immunocytologic staining with the specific antibodies.

Immunostaining was performed using the avidin–biotin complex method LSAB (HRP). After centrifugation the samples at 3000 rpm for 20 minutes, immunocytochemical labelling was performed on unstained smears. Provided the smears were well air-dried (for at least 14 hr) prior to immersion in 100% ethanol, there was no need to employ adhesive-coated glass slides. Enzymes are the most widely used labels in immunohistochemistry and incubation with a chromogen using a standard histochemical method creates colorimetric (brown) reaction (15), end-product suitable for the light microscope (NIKON Digital Camera DN100, Japan, x20, x40 si x100.) For dilution of concentrated antibody was used Antibody diluent (code S2022). Endogenous peroxidase activity was blocked using a hydrogen peroxide block (Dako REAL, Peroxidase –Bloking Solution, code S2023) for 10 min at 37°C, and then the slides were washed with distilated water and phosphate-buffered saline PBS (pH 7,4-7,6) several times afterward. The visualisation of the antibody was made with Epitope Retrieval Solution at 95-970C in incubation container, for 30 minutes. The antibody was incubated at room temperature for 30 min in a humidified environment. A polymer-labeled secondary antibody (Dako HRP) was applied for 30 min followed by incubation with 3,3`-diaminobenzidine tetrahydrochloride in organic solvent (DAB Chromogen) as a chromogen and then counterstaining with hematoxylin or Giemsa.

Visualization was achieved by using the EnVision+ peroxidase system (DAKO). Staining was considered positive only when unequivocally localized to the cell membrane in a linear pattern. Staining extent was scored on a scale of 0 through 3, as follows: score 0: staining of 0-10% cells (no staining) (-); score 1: staining of 11-40% cells (1+); score 2: staining of 41-80% cells (2+); score 3: staining of 81-100% cells (3+). Slides considered as "positive" were graded for granular cytoplasmic staining that was distinct and clearly stronger than background staining. Identifying such staining in at least 5% of tumor cells rendered the case positive. The intensity of staining was also scored on a categorical scale from 0 to 3: 0 indicated absent; 1+, very weak,

dubious staining; 2+, definite, mild, or moderate staining; 3+, definite, strong staining. Staining that was not unequivocally distinct from background staining was considered negative.

All 77 effusions were immunohistochemically analyzed for cytokeratin AE1/AE3, EMA, CEA, Calretinin, anti-human epithelial antigen, N-Cadherina, Desmina, Citokeratinele 5/6, Vimentina and CA125 expression using a manual method for staining. All antibodies were monoclonal mouse and purchased from DakoCytomation (Denmark). Details on the immunocytochemical tehnique, antibodies dilution and expression are shown in Table 1.

To estimate the accuracy of the immunocytochemical assay for diagnostic purposes, statistical analysis was performed using the two-tails unpaired t-test, determining the values of p. We considered the significant association when $p < 0.05$. The t-test was used to assess the correlation of tumour marker concentrations in serous effusion fluid and positive immunocytochemical findings. For statistical analyses, only those cases with more than 11% (1+, 2+ or 3+) of tumor cells exhibiting definite staining were considered positive. Representative images of positive staining are illustrated in Figure 1, 2, 3, 4, 5, 6.

RESULTS

Histological examination had been performed in all patients. In all cases, the origin of the neoplasm (primary site of the carcinoma) was clearly established. Clinical diagnoses and site of neoplasm associated with peritoneal effusions of all patients are detailed in Table 2.

In 85, 71% of cases the cytological diagnoses provide a certain diagnostic. The findings were recorded as "positive", "negative", or "suspicious" for the presence of malignant cells. Malignant cells were recognised morphologically in 27 of 36 (75%) specimens from patients with carcinomas, and ½ of mesotheliomas. In all other malignant cases the diagnosis of "suspicious" or "negative" was recorded; in all samples from benign diseases the diagnosis was "negative", but in 2/41 benign cases the diagnosis was "suspicious". From all 36 of effusions samples associated with carcinoma, only 27 clearly established to contain neoplastic cells on routine cytological examination. In 11 cases, the corresponding specimens were been reported to contain various uncertain reactive mezothelial cells and, in that case, the cytologic diagnosis was "suspicious for malignancies". Calculating

Table 1. Details on the immunocytochemical tehnique, antibodies dilution and expression

No.	Monoclonal antibody	Clone	Dilution	Incubation time (minutes)	Expression
1.	Pancitokeratin	AE1/AE3	1:50	20`	cytoplasmic
2.	EMA(anti-human Epithelial Membrane Antigen)	E29	Ready-to-use	30`	cytoplasmic
3.	CEA	II-7	1:25-1:5	30`	cytoplasmic
4.	Calretinin	DAK Calret 1	Ready-to-use	20`	nuclear
5.	anti-human epithelial antigen	BerEP4	1:100	30`	membrane pattern
6.	N-Cadherina	NCH-38	ready-to-use	20`	membrane pattern
7.	Desmina	D33	1:50	30`	cytoplasmic
8.	Citokeratinele 5/6	D5/16 B4	1:50	30`	cytoplasmic
9.	Vimentina	Vim 3B4	1:100	30`	cytoplasmic
10.	CA125	OC125	1:100	30`	cytoplasmic

Table 2. Cytological diagnoses and site of neoplasm associated with peritoneal effusions

Primary site of the carcinoma	Benign cases	Ovarian carcinoma	Gastrointestinal carcinoma	Breast carcinoma	Pulmonary carcinoma	Malignant mesothelioma
Cytologic diagnosis of "positive" for malignancies	0/41	15/18	7/9	2/3	2/4	1/2
Cytologic diagnosis of "suspicious"	2/41	3/18	2/9	1/3	2/4	1/2

the mean and standard deviation from the three cytologic formulas, we didn`t found any semnificative differences between them (p>0,05).

The Giemsa-stained cytological smears from benign cases were reviewed and in 2/41 samples it was possible to detect reactive mesothelial cells, bringing the suspicious of malignant disease. Therefore, the use of cytologic inspection at the incipient effusions examination not only provide in 85, 71% cases a certain diagnostic but also can detect a suspicious effusion that can be associate to a malignancy.

The immunocytochemical assessment, made by the ten monoclonal antibodies studied, on benign peritoneal effusions (no. 41) associated to non-malignant pathology had the following results: vimentin and citokeratin AE1/AE3 expression showed three patterns of positivity (1+, 2+ and 3+) for more than 50% of cases (22/41, 53,65% for AE1/AE3 and 26/41, 63,41 for vimentin) (Table 3). BerEP4, N-Cadherin and calretinin did not stain any cells in all benign effusions (percentage of negative staining 100%).

Table 3. Immunoreactions in benign cases (No.41)

Staining intensity	AE1/AE3	Calretinin	EMA	Ck5/6	CEA	N-Cadherin	Vimentin	Desmin	Ber-EP4	CA125
Score 0 (-)	19	41	40	22	39	41	15	35	41	39
Score1:(+)	9	0	0	6	0	0	12	3	0	0
Score 2:(++)	10	0	0	8	0	0	6	2	0	0
Score 3:(+++)	3	0	1	5	2	0	8	1	0	2

Table 4. Immunoreactions on peritoneal effusions associated with ovarian carcinoma (No.18)

Staining intensity	AE1/AE3	Calretinin	EMA	Ck5/6	CEA	N-Cadherin	Vimentin	Desmin	Ber-EP4	CA125
Score 0 (-)	17	18	1	8	2	16	12	17	10	0
Score1:(+)	0	0	2	0	1	0	2	0	0	4
Score 2:(++)	3	0	5	2	2	0	2	0	3	3
Score 3:(+++)	8	0	10	8	2	2	6	1	6	11

Table 5. Immunoreactions on peritoneal effusions associated with gastrointestinal carcinoma (GI) (No.9)

	AE1/AE3	Calretinin	EMA	Ck5/6	CEA	N-Cadherin	Vimentin	Desmin	Ber-EP4	CA125
Score 0 (-)	8	9	8	1	7	0	9	9	1	9
Score1:(+)	1	0	0	0	1	0	0	0	4	0
Score 2:(++)	1	0	1	0	2	2	0	0	2	0
Score 3:(+++)	3	0	0	8	4	7	3	0	2	0

Table 6. Immunoreactions on peritoneal effusions associated with breast carcinoma (No.3)

	AE1/AE3	Calretinin	EMA	Ck5/6	CEA	N-Cadherin	Vimentin	Desmin	Ber-EP4	CA125
Score 0 (-)	0	3	0	3	3	2	3	3	0	2
Score1:(+)	0	0	0	0	0	1	0	0	3	1
Score 2:(++)	2	0	2	0	0	0	1	0	0	0
Score 3:(+++)	1	0	1	0	0	0	2	1	0	0

Table 7. Immunoreactions on peritoneal effusions associated with pulmonary carcinoma (No.4)

	AE1/AE3	Calretinin	EMA	Ck5/6	CEA	N-Cadherin	Vimentin	Desmin	Ber-EP4	CA125
Score 0 (-)	3	2	1	3	1	4	4	4	2	0
Score1:(+)	0	0	0	0	2	1	0	0	3	1
Score 2:(++)	1	0	1	1	1	1	0	0	0	2
Score 3:(+++)	3	0	2	0	0	2	1	0	0	1

Table 8. Immunoreactions on peritoneal effusions associated with malignant mesotheliomas (No.2)

	AE1/AE3	Calretinin	EMA	Ck5/6	CEA	N-Cadherin	Vimentin	Desmin	Ber-EP4	CA125
Score 0 (-)	1	-	2	-	2	-	-	-	2	2
Score1:(+)	0	0	0	0	0	0	0	0	0	0
Score 2:(++)	1	0	0	1	1	0	0	0	0	0
Score 3:(+++)	1	1	0	1	0	2	2	2	0	0
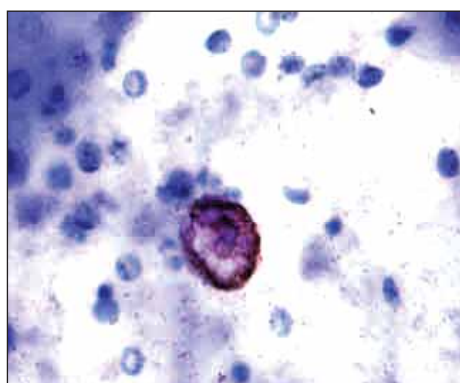
Figure 1. Metastatic breast carcinoma cell present in peritoneal effusions, positive membrane pattern immunostaining for BerEP4 (LSAB tehnique, haematoxylin counterstain, original magnifications 400x).
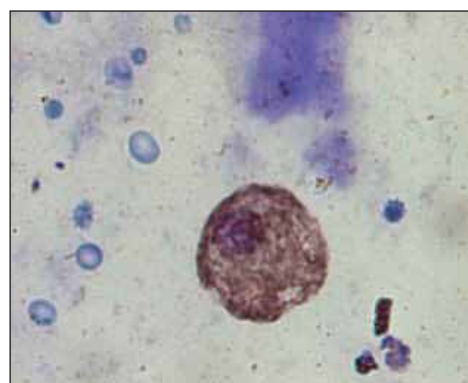
Figure 4. Positive cytoplasmic immunostaining for vimentin; large binucleate cell (LSAB tehnique, haematoxylin counterstain, original magnifications 400x).
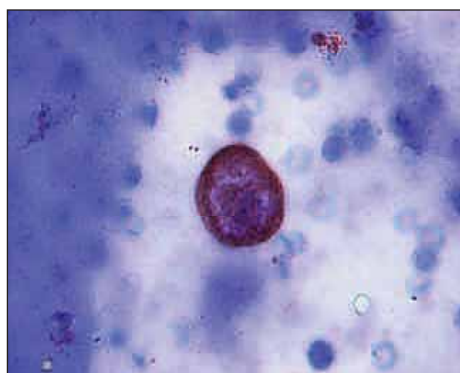

Figure 2. Immunocytochemical reactivity of monoclonal antibody N-Cadherin (positive membrane expresion) (LSAB tehnique, haematoxylin counterstain, original magnifications 400x).
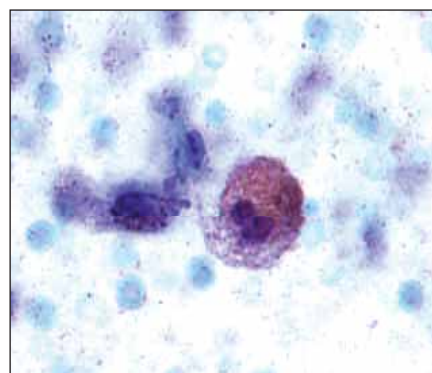

Figure 5. Diffuse moderately positive cytoplasmic immunostaining for desmin (LSAB tehnique, haematoxylin counterstain, original magnifications 400x).
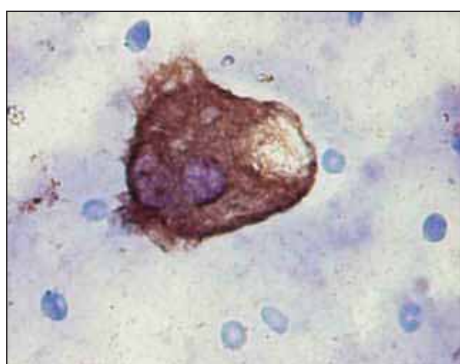

Figure 3. Positive cytoplasmic immunostaining for vimentin; large binucleate cell (LSAB tehnique, haematoxylin counterstain, original magnifications 400x).
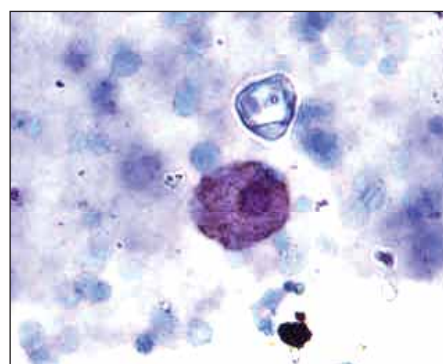

Figure 6. Immunocytochemical reactivity of monoclonalantibody CA125 (positive cytoplasmic immunostaining)(LSAB tehnique, haematoxylin counterstain, original magnifications 400x)

In two cases (considered „suspicious for malignancy" after conducting the citology method), the immunocytochemical staining had revealed more than 11% of reactive mesothelial cells labeled with each of the two monoclonal antibodies (CEA and CA125) and one were also positive for EMA. The both cases were positive in metastases or final stages, so a significant correlation was found between the expression of this mentioned markers and tumor stage (p<0,01).

Peritoneal effusions – specific immunostaining profiles using multiple markers provided high sensitivity, specificity, and positive predictive value in detecting primary carcinomas, as follows: ovarian carcinoma (CA125+/BerEP4/Desmin-/Vimentin-/N-Cadherin-/CEA-/CK5/6+/EMA+/Calretinin-/PanCKAE1/AE3-), gastrointestinal tract carcinoma (CA125 -/BerEP4+/Desmin-/Vimentin-/N-Cadherin+/CEA+/CK5/6+/EMA-/Calretinin-/ PanCKAE1/AE3-), breast carcinoma (CA125 -/ BerEP4+/Desmin-/Vimentin+/ N-Cadherin-/CEA-/CK5/6-/EMA+/Calretinin-/PanCKAE1/AE3+), pulmonary carcinoma (CA125+/BerEP4+/Desmin-/Vimentin-/N-Cadherin+/CEA+/CK5/6-/EMA+/Calretinin-/PanCKAE1/AE3-), malignant mesothelioma (CA125-/BerEP4-/Desmin+/Vimentin+/N-Cadherin+/CEA-/CK5/6+/EMA-/Calretinin+/PanCKAE1/AE3+) (Table 9).

Overall, these combined phenotypes correctly predicted the tester samples (metastatic carcinomas with known primaries) in 94,80% of cases (Table 10).

Benign peritoneal effusions can be discriminate from malignant effusions associated with ovarian carcinoma using 3 immunocytochemical markers: CA125, BerEP4 and EMA; from gastrointestinal carcinoma with BerEP4, vimentin, N-Cadherin and CEA; from breast carcinoma with BerEP4, ck5/6 and EMA; from pulmonary carcinoma with CA125, BerEP4, vimentin, N-Cadherin, CEA, ck 5/6, EMA and panck AE1/AE3

Table 9. Specific immunostaining profiles of peritoneal effusions using multiple markers

	PRIMARY SITE					
	BENIGN CONDITIONS	OVARIAN CARCINOMA	GASTROINTESTINAL CARCINOMA	BREAST CARCINOMA	PULMONARY CARCINOMA	MALIGNANT MESOTHELIOMA
CA125	-	+	-	-	+	-
BerEP4	-	+	+	+	+	-
Desmin	-	-	-	-	-	+
Vimentin	+	+	-	+	-	+
N-Cadherin	-	-	+	-	+	+
CEA	-	-	+	-	+	-
Ck5/6	+	+	+	-	-	+
EMA	-	+	-	+	+	-
Calretinin	-	-	-	-	-	+
PanCK	+	+	+	+	-	+

Table 10. Site of Origin Predicted From Immunoprofiles

Actual Primary Site	Predicted Primary Site					
	Benign conditions	Ovarian carcinoma	Gastrointestinal carcinoma	Breast carcinoma	Pulmonary carcinoma	Malignant mesothelioma
Benign conditions (41)	39	0	0	0	0	0
Ovarian carcinoma (18)	0	17	0	0	0	0
Gastrointestinal carcinoma (9)	0	1	9	0	1	0
Breast carcinoma(3)	1	0	0	2	0	0
Pulmonary carcinoma(4)	0	0	0	1	3	0
Malignant mesothelioma(2)	1	0	0	0	0	2

and from malignant mesothelioma with N-Cadherin, desmin and calretinin.

Most ovarian carcinomas can be discriminated with high probability from: gastrointestinal carcinomas using a panel of 5 antibodies directed against CA125, N-Cadherin, vimentin, CEA and EMA; from breast carcinomas using 2 antibodies directed against CA125 and ck5/6; from pulmonary carcinomas using a panel of 5 antibodies directed against N-Cadherin, vimentin, CEA, AE1/AE3 and ck5/6; from malignant peritoneal mesothelioma using a panel of 6 antibodies directed against CA125, BerEP4, desmin, N-Cadherin, EMA and calretinin.

Most gastrointestinal tract carcinomas can be discriminated with high probability from: breast carcinomas using a panel of 5 antibodies directed against N-Cadheri, CEA, ck5/6, EMA and vimentin; from pulmonary carcinoma with CA125, ck 5/6, EMA and panck AE1/AE3; from malignant mesothelioma with BerEP4, vimentin, desmin, CEA and calretinin.

Breast carcinomas can be discriminated with high probability from: pulmonary carcinomas using a panel of 5 antibodies directed against CA125, N-Cadherin, CEA, vimentin and panck AE1/AE3; from malignant mesothelioma with BerEP4, desmin, vimentin, CEA, N-Cadherin, ck 5/6, EMA , panck AE1/AE3 and calretinin.

Pulmonary carcinomas can be discriminated with high probability from malignant mesothelioma using a panel of 9 antibodies directed against CA125, BerEP4, vimentin, desmin, CEA, ck 5/6, EMA, calretinin and panck AE1/AE3.

Since the use of all monoclonal antibodies studied would require, for there frequently use in a cytopathology laboratory, quite expensive costs, we select five of the most important used markers with highest sensitivity in making a differentiation between malignant cells and reactive mezoteliale cells. These five markers, ranked in order of their sensitivity in differentiation, were: CEA, CA125, BerEP4, N-Cadherin and Calretinin.

DISCUSSION

Several immunocytochemical studies of peritoneal effusions have also been performed in patients diagnosed with different malignancys aiming to understand immune mechanisms underlying this disease. In his study, Ghosh et al. (16) used anti-CEA that gave positive reactions in 80% of carcinoma cases and was not seen to react with any other cell types. The anti-keratin reacted with malignant cells in only a small proportion of cases. The antibodies against epithe-

lial intermediate filaments reacted equally strongly with benign mesothelial cells and carcinoma cells, but gave negative reactions with lymphoma cells.

The presence of desmin was also detected in benign mesothelial cells in 47 of 56 (84%) reactive cellular specimens compared with 1 of 12 (8%) malignant mesotheliomas and 2 of 98 (2%) carcinomas. N-cadherin was expressed in 48 of 56 (86%) reactive cases, 12 of 12 (100%) mesotheliomas, and 47 of 98 (48%) carcinomas. In carcinomas, N-cadherin expression was most often seen in ovarian carcinoma but was also found in other carcinomas. Calretinin, an established marker of mesothelial cells, was detected in 52 of 56 (93%) reactive specimens, 11 of 12 (93%) mesotheliomas, and 3 of 98 (3%) carcinomas. Evaluation of staining results led to reclassification of six malignant specimens as benign, whereas 17 cases diagnosed as benign and the two diagnosed as inconclusive were classified as malignant. Desmin appears to be a promising marker for the distinction between reactive mesothelium and malignant epithelial cells in terms of both specificity and sensitivity, and its complementary use with calretinin is recommended. Unlike calretinin, it may also prove valuable for the distinction between benign and malignant mesothelial cells. N-cadherin does not have a role in the distinction between mesothelial and epithelial cells. However, it may prove useful in the characterization of carcinomas of unknown origin. As has previously been shown, a significant number of diagnoses that are based on morphologic examination alone are modified after the use of a broad antibody panel (17).

Serous effusions are common clinical syndromes and can be simply divided into benign and malignant. Differentiation between two kinds of effusions is very important for diagnosis, treatment and prognostic evaluation. The cytological examination is a simple and reliable method for the diagnosis of malignant effusions, but its sensitivity is only 40-60%(18), even lower in clinical practice. Cytological examinations are based on the cellular morphology, which may raise difficulties in distinguishing carcinoma cells from reactive mesothelial cells. Sometimes, carcinoma cells without typical morphological changes or enough number could not be diagnosed cytologically. How to identify the metastatic carcinoma cells and differentiate them from reactive mesothelial cells is key to diagnosing malignant effusions (2). In the past decade, immunocytochemistry seemed to be a valuable tool in solving the problem,

with a complementary value for cytological diagnosis of malignant effusions (19).

The diagnosis of carcinoma is important because many cases are present late and this patients may have systemic problems that prevent invasive procedures to obtain diagnostic material. The cytological features on carcinoma cells are well described, but are of little use when applied to effusion cytology for example, endothelial rimming of neoplastic cell groups and intranuclear inclusions are not notable features in an ascitic fluid.

Metastatic malignancy in peritoneal effusions, shows a relatively limited range of cytological expression, and confusion with reactive mesothelial cells can be a disturbing problem. The application of a technique which is specific to the mezothelial cell is clearly a great benefit in defining the site of origin of a neoplasm.The peritoneal effusion contained single neoplastic cells and groups of neoplastic cells showing nuclear pleomorphism and granular chromatin surrounded by moderate amounts of rather granular cytoplasm.

In this study, two benign peritoneal effusions of the 41 cases immunocytochemical staining revealed previously unrecognised malignant cells. This represents an increase in diagnostic accuracy and suggests that immunocytochemical labelling should be used routinely for the examination of cytologically negative samples from patients with suspected malignant disease. After demonstrating the presence of this immunocytochemical markers in these cells we felt that the diagnosis of carcinoma in the ascitic fluid was certain.

CONCLUSIONS

The cytopathological diagnosis of metastatic neoplasms is not always straightforward, especially in the absence of a clinical history of cancer. The usage of improved cytopathological criteria combined with immunocytochemistry may be of great diagnostic help in the identification of metastases. Conventional cytological examination furnishes much more information than the mere presence or absence of carcinoma cells. It provides evidence of the type of carcinoma; it sometimes permits the diagnosis of other neoplastic diseases and it often give clues to benign pathological processes. Hence it is not our opinion that immunochemistry can in any way replace this, but rather should be seen as a means of answering specific questions. It may also be added that the use of immunocytochemical techniques provides a means of objectively verifying the accuracy of the cytopathologist's opinion: this contribution to quality control in cytology may prove to be one of the most important results of the introduction of immunocytochemical techniques in this field. Immunochemistry can be used to increase the predictability of a cytology diagnosis if care is taken with the cytology sample preparation methodology and there is judicious use of select monoclonal antibody panels to support a specific cytology diagnosis. Up-to-date evidence-based antibody databases should be used when selecting antibody panels. This method is easy, rapid, reliable, and practical for the routine measurement of peritoneal effusions with small samples of biological material need for the analyses.

We feel that application of this specific technique of imunocitochemistry for a panel of monoclonal antibodys can confirm the diagnosis of a specific carcinoma on cytospin preparations of ascitic fluid, thus making further invasive diagnostic procedures unnecessary.

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REVISTA PRESEI MEDICALE INTERNAȚIONALE

Is aging a disease?

It's clear that the simple fact of growing older -- chronological aging -- is relentless and unstoppable. But experts studying the science of aging say it's time for a fresh look at the biological process -- one which recognizes it as a condition that can be manipulated, treated and delayed. Taking this new approach would turn the search for drugs to fight age-related diseases on its head, they say, and could speed the path to market of drugs that treat multiple illnesses like diabetes, heart disease and Alzheimer's at the same time. "If aging is seen as a disease, it changes how we respond to it. For example, it becomes the duty of doctors to treat it," said David Gems, a biogerontologist who spoke at a conference on aging in London last week called "Turning Back the Clock." At the moment, drug companies and scientists keen to develop their research on aging into tangible results are hampered by regulators in the United States and Europe who will license medicines only for specific diseases, not for something as general as aging. "Because aging is not viewed as a disease, the whole process of bringing drugs to market can't be applied to drugs that treat aging. This creates a disincentive to pharmaceutical companies to develop drugs to treat it," said Gems. The ability of humans to live longer and longer lives is being demonstrated in abundance across the world.Average life expectancies extended by as much as 30 years in developed countries during the 20th century and experts expect the same or more to happen again in this century. A study published last year by Danish researchers estimated that more than half of all babies born in wealthy nations since the year 2000 will live to see their 100th birthdays.

"THERE'S ONE THING WE'RE ALL MISSING"

But with greater age comes a heavier burden of age-related disease. Cases of dementia and Alzheimer's, incurable brain-wasting conditions, are expected to almost double every 20 years to around 66 million in 2030 and over 115 million in 2050. Diabetes, heart disease and cancer, and the cost of coping with them in aging populations, are also set to rise dramatically in coming decades in rich and poor countries alike. Nir Barzilai of the Albert Einstein College of Medicine at Yeshiva University in New York, says one way of trying to face down this enormous burden of disease is to look at the biggest risk factor common to all of them -- aging. "There's one thing everybody is missing," he said. "Aging is common for all of these diseases -- and yet we're not investigating the common mechanism for all of them. We are just looking at the specific diseases." To try to reverse that, Barzilai and many other scientists around the world are studying the genes of the very old and starting to find the genetic mechanisms, or pathways, that help them beat off the dementias, cancers, heart diseases and other age-related illnesses that bring down others who die younger. By finding the genes thought to help determine longevity, scientists think they may be able to mimic their action to not only extend life span, but, crucially, extend health span. "It is ... looking increasingly likely that pharmacological manipulation of these ... pathways could form the basis of new preventative medicines for diseases aging, and aging itself," said Andrew Dillin of the Salk Institute in California and the Howard Hughes Medical Institute. Gems says institutional and ideological barriers are standing in the way -- and a major one is the long-standing traditional view that aging is not a disease, but a natural, benign process that should not be interfered with.

CHANGING ATTITUDES?

All three experts say, however, that the ground is shifting in their direction. There is now a "groundswell" of specialists in aging, says Dillin, who are lobbying the world's biggest drug regulator, the U.S. Food and Drug Administration, to consider redefining aging as a disease in its own right. Major scientific research bodies like the U.S. National Institutes of Health and the Medical Research Council in Britain are also under pressure to put more emphasis -- and funding -- into studying how aging increases disease risk. For biogerontologists, as scientists who study the biology of aging are known, the struggle is to convince people that their goal in unpicking the science behind aging is no longer life, but healthier life. "The whole reason that we study the aging process is not actually to make people live a lot longer, it's to get people to have a more healthy lifespan," said Dillin. He sees it as a matter of re-educating the public and health authorities to see biological aging in a new light. "When we are in the public arena we tell people we're working on the aging process, the first thing they think is that we want to make a 100-year-old person live to be 250 -- and that's actually the furthest from the truth," he said. "What I want is for a 60-year-old person who is predisposed to have Alzheimer's to be able to delay that, live to be 80, and get to know their grandchildren."

Sursa: Reuters