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Moc-31, Fibronectin and CEA in the Differential Diagnosis of Malignant Effusions: An Immunocytochemical Study

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In discriminating benign and malignant origins of cytologically suspicious effusion smears a panel of antibodies against carcinoembryonic antigen (CEA), Fibronectin (F) and MOC-31 was used with immunocytochemical techniques. One hundred and thirty seven effusions were studied of which 107 had a malignant and 30 a benign aetiology as determined by clinical and histological examination. Cytologically 24 were diagnosed as benign, 97 as malignant and 14 as suspicious. Staining for F was positive in all effusions of benign and 3 of malignant origin. MOC-31 was positive in 95 (88.8%) of effusions of malignant origin but none of benign origin. Positive CEA was observed in 43% of effusions of malignant origin and in 10 of benign origin. The combination of MOC-31 positivity measured the sensitivity and specificity of the cytological examination in cases where the cytological examination result was suspicious as did F positivity improve the sensitivity for a benign origin of the effusion. Positivity or negativity for CEA is less valuable than the other parameters. (Pathology Oncology Research Vol 6, No 2, 100–103, 2000)

Keywords: MOC-31, CEA, fibronectin, immunocytochemistry, effusions, reactive atypical mesothelial cells

Introduction

The morphologic criteria used in cytology have not always ensured diagnostic accuracy especially in serous effusion smears because the differentiation between atypical reactive mesothelial cells and malignant epithelial cells can cause diagnostic difficulties.[1,9]

The application of immunocytochemical techniques may provide useful additional information and has been shown to improve the diagnostic sensitivity.[7,10,11]

MOC-31 recognizes a 40 Kd transmembrane glycoprotein of unknown function present on the membrane of epithelial and not of mesothelial cells.[12,14] Carcinoembryonic antigen (CEA) has shown promise as a tumour marker. Recent reports indicate that the expression of CEA is greater in malignant than in benign effusion smears.[5,6] Fibronectin (F), a glycoprotein of mesenchymal cells has been identified in the cytoplasm and membrane of mesothelial cells.[6]

The purpose of this study was to evaluate the usefulness of MOC-31, CEA and F staining reaction in smears of fluids submitted for routine cytologic study in distinguishing atypical reactive mesothelial cells from metastatic adenocarcinoma cells in cytologically suspicious smears.

Patients and Methods

One hundred thirty seven pleural and peritoneal effusions, submitted for routine cytologic examination were studied by immunocytochemistry. All cases were clinically and histologically diagnosed (*Table 1*). The fluids were centrifuged and smears prepared from the pellet. For Papanicolaou staining smears were fixed in ethanol (95%) and air dried smears were prepared for Giemsa staining. For immunocytochemical study all smears were air dried, fixed for 10 min in cold acetone (–10°C) and stored at –70°C until use.

Immunocytochemical staining was performed by the Avidin-Biotin Complex (ABC) immunoperoxidase method.[4] Smears were incubated for 45 min with normal

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horse serum diluted 1:50 in PBS (Dakopatts, Glostrup, Denmark) to reduce background staining. The primary antibodies CEA (1:100 dilution) and F (1:200 dilution) (Dakopatts, Glostrup, Denmark) and MOC-31 (1:40 dilution) (Eurodiagnostic, Apeldoorn, Netherlands) were incubated for 40 min followed by incubation with the secondary antibody for 30 min and an avidin-biotin-peroxidase complex for 30 min. Peroxidase enzyme staining was achieved by incubation with 3-amino-9-ethyl carbazole and hydrogen peroxidase for 10 min. Between incubations the smears were rinsed with phosphate bufferd saline solution (0.05 µl/L, pH 7.6). Smears were counterstained with Mayer's hematoxylin and covered with mounting medium (Glycergel, Dako, Glostrup, Denmark). Control slides were incubated with phosphate buffered saline solution.

The routine Papanikolaou and Giemsa stained smears were classified as positive when cells defined as malignant were found, as suspicious when atypical cells were found and as negative when reactive mesothelial cells or no malignant cells were observed. The final cytologic diagnosis was established after combining the results of the cytomorphologic diagnosis and the immunocytochemical staining reaction. The histological and clinical diagnoses were used as the standard diagnosis.

Immunocytochemical reactivities were evaluated by calculating the proportion of positively stained cells in at least 10 visual fields. The intensity of staining was scored on a four point scale: 0 = no staining, 1 = weak but unequivocal staining, 2 = definite staining of moderate intensity, 3 = strong staining. Only tumour cells scoring 2 or more were considered positive, regardless of the num-

Table 1. **Data of the examined fluids**

Histologic or clinical diagnosis of the studied cases	*Effusion*	*No of cases*
Benign diseases		
Viral pleuritis	Pleural	6
Cardiac failure	Pleural	5
Pulmonary embolism	Pleural	2
Tuberculosis	Pleural	5
Liver cirrhosis	Peritoneal	5
Peritonitis	Peritoneal	2
Renal failure	Peritoneal	3
Hepatitis	Peritoneal	2
Total		*30*
Malignant diseases		
Ovarian adenoCa	Peritoneal	24
Ovarian adenoCa	Pleural	8
Breast adenoCa	Pleural	47
Breast adenoCa	Peritoneal	5
Lung adenoCa	Pleural	7
Stomach adenoCa	Peritoneal	5
Colon adenoCa	Peritoneal	3
Endometrial adenoCa	Peritoneal	4
Total		*107*

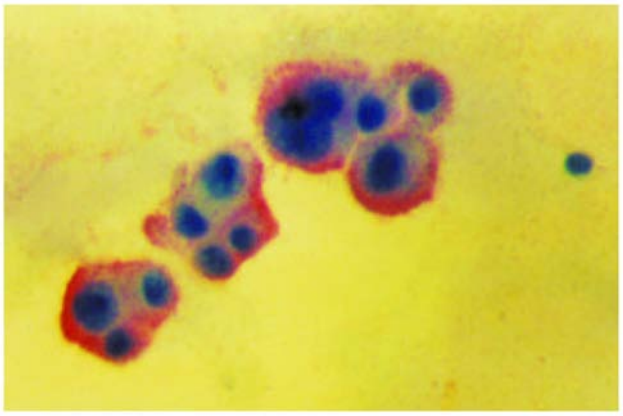

Figure 1. *Pleural fluid: Clusters of atypical reactive mesothelial cells from a case of viral pleurity showing positivity for Fibronectin (X500).*

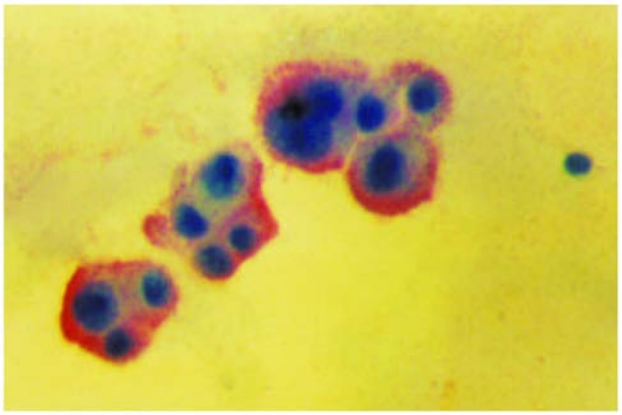

Figure 2. *Peritoneal fluid: Malignant ovarian adenocarcinoma cells with positive reaction for MOC-31 (X500).*

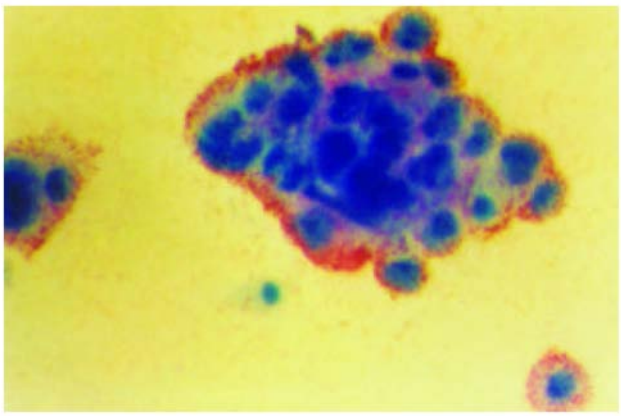

Figure 3. *Pleural fluid: A cluster of malignant breast adenocarcinoma cells showing positivity for CEA (X500).*

Table 2. **Cases cytologically diagnosed as suspicious for malignancy**

Histological or clinical diagnosis	*No of cases*
Benign diseases	
Cirrhosis	1
Viral pleuritis	1
Pulmonary embolism	2
Renal failure	1
Total	*5*
Malignant diseases	
Ovarian adenoCa	5
Breast adenoCa	3
Total	*8*

Table 3. **Result of immunocytochemical staining for F, MOC-31 and CEA in 4 groups selected according to their cytological and histological diagnosis**

	(CB)(HB)	*(CM)(HM)*	*(CS)(HB)*	*(CS)(HM)*
Total	25	99	5	8
F+ve	25	1	5	2
MOC-31+ve	0	90	0	5
CEA+ve	1	43	2	3

C=Cytological B=Benign S=Suspicious
H=Histological M=Malignant

ber of cells stained *(Figure 1,2,3).* Sensitivity and specificity of F, CEA and MOC-31 and their combinations in the cytologically benign, suspicious and malignant groups were calculated. Sensitivity and specificity of the 3 substances alone or in combination with cytology were determined.

Results

Of the 137 effusion smears 107 were shown histologically to be malignant and 30 benign. *Table 1* shows the etiology of the effusions. Cytologically 99 were malignant, 25 were benign and 13 were suspicious. The histological or clinical diagnosis and aetiology of the 13 cytologically suspicious effusion smears. Eight malignant and 5 benign are shown in *Table 2.* Of the 107 histologically malignant effusions 95 had MOC-31 positive smears, 46 CEA positive and only 3 F positive. None of the 30 histologically or clinically benign effusions had MOC-31 positive smears, 3 were CEA positive but all were F positive. Thus MOC-31 has a sensitivity of 88.7% for malignancy and as none of the benign smears were MOC-31 positive a specificity of 100%. The sensitivity of CEA positivity for malignancy was 43% and the specificity 90%. Positivity of the antigen F was 100% sensitive and 96% specific for a benign etiology. A negative MOC-31 is 100% sensitive and 88% specific and a negative CEA 90% sensitive and 43% specific for a benign aetiology. In no case was a cytologically malignant or benign smear shown to be benign or malignant on histological examination. Thus for the further clarification of the cytologically suspicious smears four groups were separated for separate study. These are shown in *Table 3* together with the results of the immunocytochemical staining for F, MOC-31 and CEA. From the table it can be seen that of the eight cytologically suspicious smears which proved histologically to be malignant, the 5 were MOC-31 positive. Three of these were also CEA positive. Of the 3 which remained 2 were F positive and 1 was negative for all three antigens. Thus 62% of cytologically suspicious but histologically malignant effusions could be correctly classified using MOC-31 in combination with the cytological analysis increasing the sensitivity of cytological analysis alone to 97% from a value of 92% for cytological analysis by itself without reduction of the 83% specificity of the cytological diagnosis.

The combination of cytology and CEA positivity as an indication of malignancy has a 95% sensitivity for malignancy but reducing the specificity to 73% in comparison to 83% of the cytological analysis alone. In a similar manner combining cytological analysis with F positivity as an indication of a benign aetiology raises the sensitivity to 100% and reduces the specificity to 89%. These values are shown in *Table 4.*

Discussion

Immunocytochemical techniques have now become widely used in cytopathology for the demonstration of a large number of various antigens in effusion smears as an aid in differentiating malignant adenocarcinoma cells from reactive atypical mesothelial cells.[6,8,15]

Table 4. **The effect of combining cytological analysis with the results of immunocytochemical staining on the sensitivity and specificity of cytological analysis alone in the determination of malignant and benign effusions**

	Sensitivity (%)	*Specificity (%)*
Malignant etiology		
Cytology alone	92.5	83
Cytology – MOC-31$^+$	97	83
Cytology – CEA$^+$	95	73
Benign etiology		
Cytology	83.3	92.5
Cytology – F$^+$	100	89
Cytology – CEA$^+$	90	43

In the present study the group of effusions to which the greatest attention was given is that in which the gold standard fails, which in this case is the suspicious group. This problem was the fuel to apply in our study the immunocytochemical technique with the use of a pannel of antibodies (F, MOC-31 and CEA) in effusion smears.

According to many investigators F and CEA have higher specificities for benign and malignant cells (90% and 92.5% respectively). The percentage of carcinomas with positive reaction for CEA varied from 48% to 96% in studies.[4,6,13] Moreover MOC-31 is an antibody which can play an important role in the identification of metastatic adenocarcinoma cells in effusion smears.[2,12,14]

Cytological examination of effusion smears is a valuable tool in the diagnosis of the aetiology of the effusions. In no case in our material was a cytologically benign or malignant effusion found to be associated with a histologically malignant or benign lesion. There were however 13 suspicious smears and the results obtained from this study are a strong indication that immunocytochemical staining can provide valuable additional diagnosis information in these cases. In our study cytological examination was 92.5% sensitive for malignancy, MOC-31 positivity 88.7% sensitive and the combination 97% sensitive for a malignant aetiology. CEA positivity was 43% sensitive for a malignant aetiology but combining cytology with CEA positivity mereared the sensitivity to 95%. In a similar manner cytology has an 83.3% sensitivity for a benign aetiology, fibronectin positivity 100% sensitivity and the two together a 100% sensitivity for a benign aetiology. It should be noted that the „combinations" include these cases where the cytology was suspicious. Cytology in combination with CEA negativity, which by itself is 90% sensitive for a benign aetiology, also has a 90% sensitivity for a benign aetiology. The problem with the latter combination however is its low specificity of 43% which means that it is probably of not much real use in these cases where the cytology is suspicious.

It can be therefore stated that the results of this study demonstrate that F positivity in a cytologically suspicious smear is a very strong indication (89% specificity) that the aetiology of the effusion will prove to be benign, MOC-31 positivity in a cytologically suspicious smear is almost as strong as indicator (specificity 83%) that the effusion will be of malignant aetiology. Positivity for CEA antigen is a less strong indication (specificity 73%) of malignant etiology and a negative CEA in a suspicious cytological is probably not (specificity 43%) a reliable indicator of benign aetiology. The use of immunocytochemical staining for MOC-31 and Fibronectin in smears which are cytologically suspicious for malignancy provide valuable additional diagnostic information. When the routine cytological examination is sured definitely as benign or malignant these techniques can only have a confirmative role to play.

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