Virchows Arch A (1987) 411:299–304

Virchows Archiv A

Distinction between cells in serous effusions using a panel of antibodies

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Summary. In serous effusions the distinction between reactive mesothelial cells and malignant cells (especially adenocarcinoma cells and malignant mesothelial cells) is frequently a cause of diagnostic difficulty. The present paper describes the immunocytochemical staining of cells in 76 effusions from 71 patients with different malignancies. In 91% of the effusions obtained from patients with adenocarcinomas, the cells stained positive for anti-EMA, 94% for anti-CEA, 64% for anti-LeuM1-antigen and 75% for anti-keratins. In more than 90% of the cases the reactive mesothelial cells stained positive for anti-keratins, but not for anti-EMA, anti-CEA or anti-LeuM1-antigen. It is concluded that a panel of the antibodies against EMA, CEA, LeuM1-antigen and keratins is valuable in the distinction between adenocarcinoma cells, malignant mesothelial cells and reactive mesothelial cells in serous effusions.

Key words: Serous effusion – Immunocytochemistry – Adenocarcinoma cells – Reactive mesothelial cells – Malignant mesothelial cells

Introduction

In serous effusions the distinction between reactive mesothelial cells and malignant cells, (especially adenocarcinoma cells and malignant mesothelial cells) is frequently a cause of diagnostic difficulty. However, previous studies have shown that the use of immunocytochemistry is of value in differential diagnosis, especially using antibodies against Carcinoembryonic antigen (CEA). Antibodies against Epithelial membrane antigen (EMA), keratins and a number of other antibodies have also been suggested (To et al. 1981; Kahn et al. 1982; Sehested et al. 1983; Walts et al. 1983; Walts et al. 1984; Chess et al. 1986).

Recently the myelo-monocyte monoclonal antibody against LeuM1-antigen has been reported to react with a majority (54–84%) of lung adenocarcinomas in formalin fixed paraffin-embedded histological sections (Sheibani et al. 1986).

In the present paper the application of antibodies against EMA, CEA, LeuM1-antigen, keratins and leucocyte common antigen (LC) to serous effusions is reported in order to aid in the differentiation between adenocarcinoma cells, malignant mesothelial cells and reactive mesothelial cells.

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Materials and methods

Seventy-six effusions, submitted for routine cytological examination were studied obtained from 71 patients with various malignant diseases. The specimens included 16 ascitic and 60 pleural fluids. The distribution of the malignant disease appears in Table 1. All the specimens were submitted unfixed. Cytospins were made using the Shandon Elliott cytocentrifuge. A few ul were centrifuged at 1500 rpm. for 10 min and the sediment was placed onto clean glass slides and air dried. The rest of each effusion was centrifuged, and the sediment was prepared for paraffin sections as described elsewhere (Sehested et al. 1983). The cytospins were methanol fixed and stained according to the Papanicolaou and May-Grünvald-Giemsa methods. The paraffin sections were stained with haematoxylin-eosin and van Gieson-Alcian Blue. In each case the same panel of antibodies was applied to both the cytospins and the paraffin sections.

The antibodies used for characterization of the cells are listed in Table 2. The exact characterization of the antibodies has been described elsewhere (O'Brien et al. 1980; To et al. 1981; Kahn et al. 1982; Corson et al. 1983; Kurtin et al. 1985; Said 1983; Walts et al. 1984; Jack et al. 1986; Sheibani et al. 1986).

Immunocytochemical staining was performed by using immune complexes of alkaline phosphatase and monoclonal anti-

Table 1. The distribution of the malignant diseases, and the results of the cytomorphological evaluation on serous effusions

Histological diagnosis (Biopsy verified)	Number of effusions	Number of effusions with positive cytomorphology	Number of effusions with suspicious cytomorphology	Number of effusions with negative cytomorphology
Mammary carcinomas	22	8	3	11
Ovary carcinomas	18	5	9	4
Lung carcinomas				
small cell anaplastic carcinomas	9	4	0	6
adenocarcinomas	12	6	5	1
large cell carcinomas	2	1	0	0
Mesotheliomas	3	3	0	0
Gastric adenocarcinomas	2	2	0	0
Colonic adenocarcinomas	2	2	0	0
Endometrial adenocarcinomas	2	2	0	0
Cervix carcinomas		0	0	1
Esophageal carcinomas	1	0	0	1
Hairy cell leukaemia	1	0	0	1
Malignant lymphoma	1	0	0	1
Totals	76	33	17	26

Table 2. Antibodies used for immunocytochemical characterization of cells in effusions

Antibody	Specificity against	Dilution	Source
EMA (monoclonal)	Carcinomas, malignant mesotheliomas	1:10	DAKO
CEA (polyclonal)	Adenocarcinomas	1:200	DAKO
LeuM1 (monoclonal)	Granulocytes, monocytes, Reed-Sternberg cells	1:20	Becton-Dickinson
Keratin (polyclonal) Subunits	Mesothelium, carcinomas 48, 51, 52, 56, 58, 60, kD	1:200	DAKO
LC (monoclonal)	Leucocytes, macrophages	1:5	DAKO

alkaline phosphatase (APAAP) for both the cytospins and the paraffin sections (Cordell et al. 1984).

Microscopic evaluation. The routine preparations were graded as "positive" when cells defined as malignant were discovered, "suspect" when uncertainty existed as to whether cells were carcinoma cells or reactive mesothelial cells and "negative" when no carcinoma cells were observed. The result of the cytomorphological evaluation appears from Table 1. The immunocytochemical staining was regarded as positive when staining localized in the cytoplasm as well as on the cell membrane was observed. In the mesotheliomas, the diagnosis was histologically confirmed on pleural biopsies.

Results

In all effusions the number of immunostained cells varied from a few scattered to virtually all cells. All controls were negative.

Immunocytochemical staining for EMA produced a strong "rim" pattern or a moderate degree of a diffuse staining reaction of the cytoplasm in the carcinoma cells (Fig. 1) and occasionally a weak diffuse cytoplasmatic staining of the reactive mesothelial cells. Malignant mesothelial cells revealed a strong diffuse cytoplasmatic staining reaction. The staining reaction was the same in both the cytospins and the paraffin sections.

Staining for CEA produced a strong diffuse cytoplasmatic reaction in adenocarcinoma cells in both the cytospins and the paraffin sections (Fig. 2). No reactive or malignant mesothelial cells were stained.

Staining for LeuM1-antigen produced a strong diffuse reaction for granulocytes and a strong diffuse staining for blood macrophages in the cytospins but not in the paraffin sections. The adenocarcinoma cells produced a variable degree of focal or diffuse, but intensely positive cytoplasmatic immunostaining in both the cytospins and the paraffin sections (Fig. 3). No reactive or malignant mesothelial cells were stained.

Staining for keratins produced a weak diffuse or peripheral staining reaction for the adenocarcinoma cells (Fig. 4) and a strong perinuclear and sometimes peripheral staining reaction for reactive

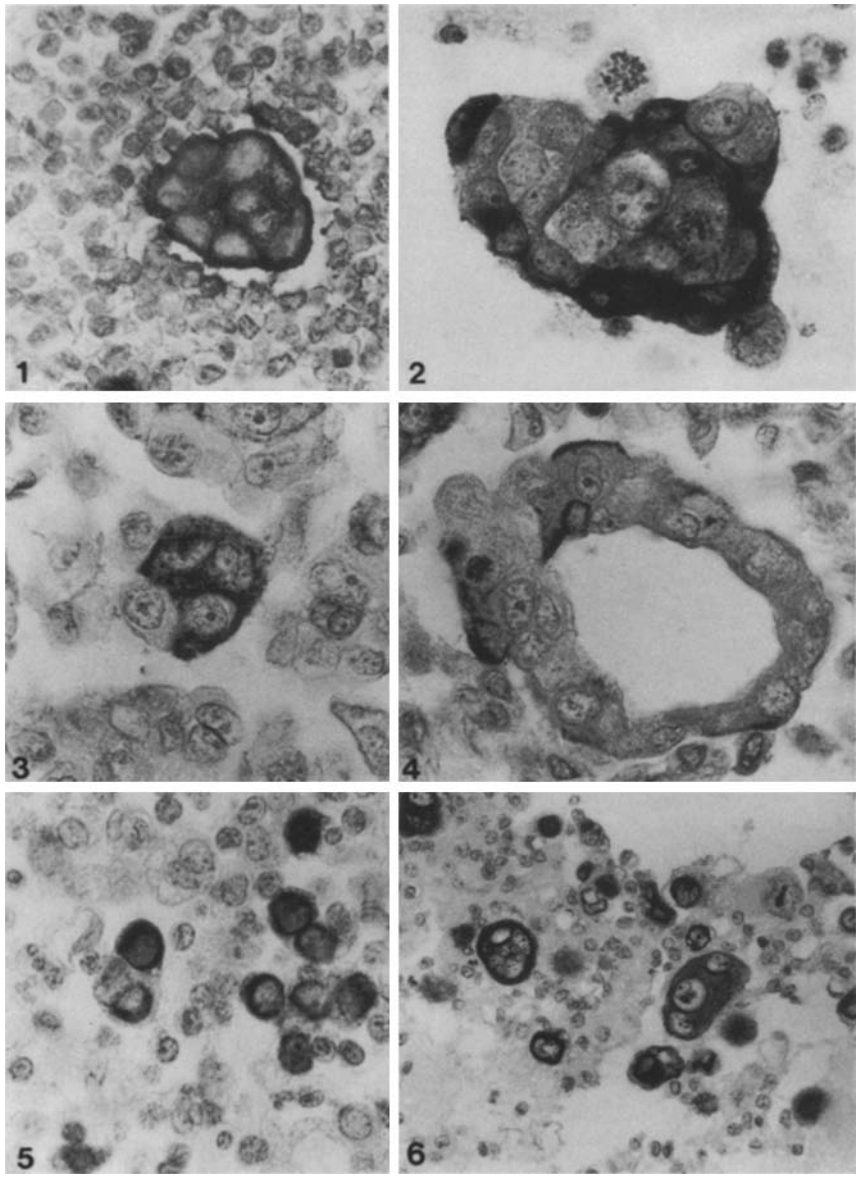

Fig. 1. Diffuse cytoplasmatic immunostaining for EMA in adenocarcinoma cells in paraffin sections (×900)

Fig. 2. Diffuse cytoplasmatic immunostaining for CEA in adenocarcinoma cells in paraffin sections (×800)

Fig. 3. Strong diffuse cytoplasmatic immunostaining for LeuM1-antigen in adenocarcinoma cells in paraffin sections (×800)

Fig. 4. Weak peripheral immunostaining for keratins in adenocarcinoma cells in paraffin sections (×800)

Fig. 5. Strong perinuclear immunostaining for keratins in reactive mesothelial cells in paraffin sections (×800)

Fig. 6. Strong diffuse cytoplasmatic immunostaining for keratins in malignant mesothelial cells in paraffin sections (×320)

mesothelial cells (Fig. 5). The peripheral staining reaction was most evident in the cytospins. In malignant mesothelial cells the staining was strong and diffuse in both the cytospins and the paraffin sections (Fig. 6).

Staining for LC produced a strong membrane reaction for most lymphocytes and a variable degree of membrane or cytoplasmatic staining for blood macrophages. The reaction was the same in both the cytospins and the parafin sections. No reactive mesothelial or tumour cells were stained.

In effusions from patients with "positive" cytomorphology, a group of 33 effusions, 22 contained cells with positive staining for EMA, 23 contained cells with positive staining for CEA, 12 contained cells positive for LeuM1-antigen, 16 contained cells with a weak diffuse or peripheral staining for keratins, and 2 contained cells with a strong diffuse cytoplasmatic staining for kerall cell anaplastic tumour cells did not react with any of the antibodies. In these effusions 31 contained cells with a strong perinuclear staining for keratins. These cells were considered as reactive mesothelial cells.

Of 17 effusions from patients with "suspicious" cytomorphology 12 contained suspicious cells with positive staining for EMA, CEA and LeuM1, as well as a weak diffuse or peripheral staining for keratin. These cells were subsequently considered to be carcinoma cells. In the remaining

Table 3. Result of immunocytochemical staining for EMA, CEA, LeuM1-antigen, keratins and LC on serous effusions containing tumour cells

Histological diagnosis	Number of effusions	Number of effusions containing tumour cells	EMA positive	CEA positive	LeuM1 positive	Keratin positive	LC positive
Mammary carcinomas	22	11	9	10	3	7	0
Ovary carcinomas	18	9	9	8	7	7	0
Lung carcinomas							
small cell anaplastic carcinoma	9	4	0	0	0	0	0
adenocarcinomas	12	11	10	11	8	8	0
large cell carcinomas	2	1	1	1	0	1	0
Mesotheliomas	3	3	2	0	0	2	0
Gastric adenocarcinomas	2	2	2	2	2	2	0
Colonic adenocarcinomas	2	2	2	2	2	2	0
Endometrial adenocarcinomas	2	2	2	2	2	2	0
Cervix carcinomas	1	0					
Esophageal carcinoma	1	0					
Hairy cell leucaemia	1	0					
Malignant lymphoma	1	0					
Total number of adenocarcinomas	58	37	34 (91%)	35 (94%)	24 (64%)	28 (75%)	0 0
Mesotheliomas	3	3	2	0	0	2	0
Other malignancies	15	5	1	1	0	1	0

Table 4. Result of immunocytochemical staining for EMA, CEA, LeuM1-antigen, keratins and LC on reactive mesothelial cells in the total number of effusions

Histological diagnosis	Number of effusions	Number of effusions containing reactive mesothelial cells	EMA positive	CEA positive	LeuM1 positive	Keratin positive	LC positive
Mammary carcinomas	22	22	2	0	0	21	0
Ovary carcinomas	18	18		0	0	16	0
Lung carcinomas							
Small cell anaplastic carcinoma	9	9	1	0	0	9	0
adenocarcinoma	12	12		0	0	11	0
large cell carcinoma	2	2		0	0	2	0
Mesotheliomas	3	3		0	0	0	0
Gastric adenocarcinomas	2	2	1	0	0	2	0
Colonic adenocarcinomas	2	2		0	0	2	0
Endometrial adenocarcinomas	2	2		0	0	2	0
Cervix carcinomas	1	1		0	0	1	0
Esophageal carcinomas	1	1		0	0	1	0
Hairy cell leucaemia	1	1		0	0	1	0
Malignant lymphoma	1	1		0	0	1	0
Totals	76	76	4	0	0	69	0

5 effusions the suspicious cells did not stain for EMA, CEA or LeuM1, while the staining for keratins produced a strong perinuclear staining reaction. These cells were subsequent considered as benign reactive mesothelial cells.

In the total number of effusions containing cells with "suspicious" cytomorphology, 14 contained cells considered to be reactive mesothelial cells, because they revealed a strong perinuclear staining reaction for keratins. The result of the staining reaction of the tumour cells appears from Table 3 and the result of the staining reaction for reactive mesothelial cells appears from Table 4. There was no "false negative".

The group of effusions from patients with "negative" cytomorphology comprised 26 cases. 24 effusions contained cells with a strong perinuclear staining reaction for keratins. Of these cases, 5 contained cells with a weak peripheral cytoplasmatic immunostaining most evident in the cytospins. Four effusions contained a few cells with a weak diffuse cytoplasmatic staining reaction for EMA. No cells with staining reaction for CEA or LeuM1-antigen were seen. The blood macrophages produced positive staining reaction for LeuM1-antigen and LC as mentioned above.

Discussion

The purpose of this study was to compare the immunocytochemical reactivity pattern of a number of mono- and polyclonal antibodies in exfoliated cells in serous effusions and to assess their potential value in routine diagnostic cytology with special reference to differentiation between reactive mesothelial cells, adenocarcinoma cells and malignant mesothelial cells.

The results of the present study are comparable with those reported previously. However, the immunoperoxidase technique has been used in most of the previous studies (Sehested et al. 1983; Walts et al. 1983; Walts et al. 1984; Chess et al. 1986). The APAAP technique used in this study did not exert an influence on the results.

The staining reaction of the different cell types were as follows:

Adenocarcinoma cells consistently gave a variable diffuse cytoplasmatic but most often a strong "rim" pattern staining reaction for EMA in 91% of the cases and a strong diffuse cytoplasmatic staining for CEA in 94% of the cases. In 64% of the cases the adenocarcinoma cells exhibited a variable degree of focal or diffuse but intense cytoplasmatic immunostaining for anti-LeuM1-antibody. In 75% of the cases the cells expressed a weak peripheral or diffuse cytoplasmatic staining for keratins. None of the adenocarcinoma cells were positive for LC.

Malignant mesothelial cells expressed a strong diffuse cytoplasmatic immunostaining for EMA and a strong diffuse cytoplasmatic staining for keratins in 2 out of 3 cases. There was no reaction for CEA, LeuM1 or LC.

Small cell anaplastic tumour cells did not react with any of the antibodies.

Reactive mesothelial cells expressed a strong perinuclear and sometimes peripheral immunostaining for keratins in more than 90% of the cases (Table 4). The peripheral staining reaction was most evident in the air dried cytospins. Occasionally, the EMA produced a weak diffuse staining. None of the reactive mesothel cells expressed CEA, LeuM1 or LC.

However, some authors have been able to demonstrate a weak or moderate expression of CEA in reactive mesothelial cells (Corson et al. 1982), but most authors have not been able to confirm this (Sehested et al. 1983; Walts et al. 1983; Chess et al. 1986).

While the blood macrophages only expressed LeuM1-antigen in the cytospins, the blood macrophages reacted with LC in the cytospins as well as in the paraffin sections.

The anti-LeuM1-antibody which is expressed by the neoplastic cells of patients with Hodgkin's disease has recently been described to stain 58–94% of adenocarcinomas in paraffin embedded histological sections, primarily lung adenocarcinomas, but in neither malignant nor benign mesothelium (Sheibani et al. 1986).

The expression of LeuM1-antigen in exfoliated adenocarcinoma cells in serous effusions has not been reported before. In this study 64% of the cases of the total number of adenocarcinoma cells expressed LeuM1-antigen, primarily lung adenocarcinoma cells and ovary carcinoma cells (72% and 77% respectively). Only in three cases (27%) did the mammary carcinoma cells express LeuM1-antigen. Neither reactive nor malignant mesothelial cells were stained.

The use of antibodies against keratin filaments of different molecular weights have been used to differentiate between cells in serous effusions. Walts et al. have shown that malignant mesothelial cells contain high molecular weight keratin (>60 kD) in contrast to adenocarcinoma cells which contain low molecular weight keratins (45–46 kD). However reactive mesothelial cells contain keratins of different molecular weights (45, 46, 55, 63 kD). The staining pattern in this study was the same as previous described (Walts et al. 1984; Kahn et al. 1982).

In conclusion, although the immunocytochemical techniques cannot replace conventional analysis of cytological specimens of serous effusions, the present study indicates the following: 1) the use of a panel of antibodies against EMA, CEA, LeuM1-antigen, keratin and LC may finally characterize morphological suspicious cells with special regard to differentiation between reactive mesothelial cells, adenocarcinoma cells and malignant mesothelial cells, 2) the use of the antibodies may confirm the result in positive and negative cases.

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Accepted March 11, 1987