Immunocytochemical staining with monoclonal antibodies in cytologically "negative" serous effusions from patients with malignant disease

AK GHOSH,* DY MASON,* AI SPRIGGS†

From the *Nuffield Department of Pathology, University of Oxford and the †Laboratory of Clinical Cytology, Churchill Hospital, Oxford.

SUMMARY In order to assess the practical value of immunocytochemical techniques in routine cytological diagnosis, a panel of three monoclonal antibodies (anti-CEA, Ca 1 and HMFG-2) were used to search for malignant cells in 53 samples of pleural or peritoneal fluid from 41 selected patients. All these patients suffered from malignant disease, but neoplastic cells had not been reported on conventional cytological examination. In 12 of the 41 cases immunocytochemical staining revealed previously unrecognised malignant cells. This represents an increase in diagnostic accuracy of approximately 20% and suggests that immunocytochemical labelling should be used routinely for the examination of cytologically negative samples from patients with suspected malignant disease.

Effusions complicating malignant disease frequently contain easily recognisable neoplastic cells. In other cases, however, malignant cells are not seen or else their identification is too doubtful to justify a positive report.

One means by which diagnostic accuracy may be increased in such cases is to use immunocytochemical techniques. Several laboratories have explored this approach (see references in preceding paper)¹ and have reported that neoplastic cells in serous effusions can be identified in this way without difficulty. The most important question is whether these methods can reveal tumour cells in samples which otherwise would be reported "negative" for malignancy.²

We have therefore analysed a series of serous effusion samples, none of which had been reported to contain neoplastic cells on routine cytological examination, but which all came from patients shown to have malignant disease. The aim of this study was to decide whether immunocytochemical techniques have a practical role to play in the routine diagnosis of cytological samples.

Material and methods

PATIENTS AND SAMPLES (TABLE 1)

All samples in this study came from patients who had been documented as having malignant disease

Accepted for publication 21 June 1983

on the basis of clinical evaluation together with radiological studies, surgical biopsy and/or necropsy. In all but two cases the primary site of the carcinoma was clearly established, although histological examination had not been performed in all patients. Serous effusion samples from these patients had been received in the Clinical Cytology Department of the Churchill Hospital, Oxford. Smears of cell deposits from these samples were prepared and stained by routine procedures using May-Grunwald-Giemsa and Papanicolaou stains. Immunocytochemical labelling was performed on unstained smears which had been stored at -20°C for periods of up to 18 months.

MONOCLONAL ANTIBODIES

Antibody Ca 1 was kindly provided by Wellcome Diagnostics. This antibody, raised by Ashall *et al.*,³ is directed against a cancer-associated antigen which has been detected on a range of different types of human neoplasms.⁴ Its use for the detection of malignant cells in serous effusions has been described previously.¹ The antibody was obtained as a lyophilised preparation of tissue culture supernatant. After reconstitution it was used at a dilution of 1/2.

Anti-CEA antibody was kindly provided by Dr JRF Corvalan. The reactivity of this antibody with normal and malignant gastrointestinal epithelium (colon and stomach) and with breast carcinomas in

Table 1 Site of malignancy and type of effusion of 41 patients

Primary	Pleural	Peritoneal
Breast	8	2
Ovary	0	4
Endometrium	0	1
Lung	14	1
GI tract	1	5
Prostate	2	0
Hepatoma	0	1
Unknown site	1	1
Total	26	15

cyrostat sections has been documented previously.⁶ The antigen has also been demonstrated on malignant cells in serous effusions from carcinomas of breast, ovary, lung, stomach and colon.¹ The antibody was received as ascites and was used at a dilution of 1/10 000.

Antibody HMFG-2 was kindly provided by Dr J Taylor-Papadimitriou. This antibody is directed against a determinant found in the membrane of human milk fat globules and also on a variety of carcinomas including breast, ovary, lung and colon.⁶ The antigen is also expressed, more weakly, on some normal epithelial cells. The reactions of this antibody with cells from serous effusions has been described in the preceding paper.¹ The antibody was obtained as tissue culture supernatant and was used at a dilution of 1/5.

IMMUNOCYTOCHEMICAL STAINING TECHNIQUE
Details of the immunoalkaline phosphatase procedure used for staining cytological smears are given in the preceding paper.¹

Results

All but one of the cytological smears analysed by the immunoalkaline phosphatase technique gave satisfactory results, but one specimen was excluded because of non-specific staining. Effusions could be divided on the basis of their staining into three categories (Table 2):

(i) Negative result (19 patients)

No evidence of malignancy could be obtained by immunocytochemical staining in this group (which comprised 24 specimens). No positively stained cells were seen in any of the smears stained with anti-CEA or Ca 1. In three samples weak staining was obtained with antibody HMFG-2, but all positive cells in these smears had the appearance of benign mesothelial cells.

In four of these 19 patients the subsequent course showed that the effusions were unrelated to the previous (resected) cancer, since they resolved spontaneously and the patients now appear well. Three of these patients had carcinoma of the breast, and one carcinoma of the prostate. In one other case, a peritoneal effusion after resection of carcinoma of the rectum was found to be due to obstruction from adhesions, and not to metastases.

(ii) Positive result (12 patients)

In each of these cases, providing in all 15 specimens, at least two of the antibodies gave positive staining, and there were at least five stained cells which were morphologically consistent with carcinoma cells. The original Giemsa-stained cytological smears from these cases were reviewed and in three samples it was possible to detect clearly malignant cells

Table 2 Immunocytochemical diagnosis of 53 effusions from 41 patients with malignant disease and negative cytology

Primary site	No	Negative	Positive			Inconclusive				
		(No of cases)	(No of cases)	Ca 1	CEA	HMFG-2	(No of cases)	Ca 1	CEA	HMFG-2
Breast	10	3 ^a	3 ^a	+	+	+	1	-	-	+
			1	+	-	+				
			1 ^b	-	+	+				
			1 ^b	+	+	-				
Ovary	4	2	1	+	+	+	1	-	-	+
Endometrium	1	0					1 ^{aa}	+	+	+
Lung	15	10 ^{ab}	1 ^a	+	+	+	2	+	+	-
			1	-	+	+	1	-	+	+
GI tract	6	3	2	+	+	+	1 ^a	+	-	-
Prostate	2	1 ^a					1	-	-	+
Hepatoma	1	0					1	+	-	+
Unknown site	2	0	1	+	+	+	1	+	-	+
Totals	41	19	12				10			

Patients with more than one effusion gave similar results and are entered as a single case in these groups

a = patients with two effusions } each letter represents a patient.
b = patients with three effusions }

which had been missed. These three specimens were all from cases in which immunocytochemical staining had revealed more than 20 neoplastic cells labelled with each of the three monoclonal antibodies.

In one effusion, due to carcinoma of the colon, the labelled cells corresponded to a cell type subsequently shown to secrete mucin (amylase-resistant PAS-positive staining in the distended cytoplasm). In two other cases, malignant cells were reported on routine examination of preceding or subsequent effusions, but not on the one used for this study.

(iii) Inconclusive result (10 patients)

In these cases, comprising a total of 13 samples, immunocytochemical staining revealed less than five positively reacting cells in any individual smear. In eight of them the morphology of these cells seemed on review to be consistent with malignancy. One patient with carcinoma of the lung had single cells positive with anti-CEA and Ca 1 in each of two effusions. The cells had features of oat cells but were too few for a conclusive diagnosis.

In two cases the evidence was even weaker. One case of carcinoma of the prostate showed cells staining strongly with HMFG-2, but not with Ca 1 or anti-CEA. The other case, with carcinoma of the colon, showed only one weakly Ca 1-positive cell.

The "inconclusive" group therefore varies from cases in which the evidence is very suggestive but not compelling, down to those in which it depends upon the reaction of a single cell.

Discussion

Pleural, peritoneal and pericardial effusions are commonly encountered in patients suffering from malignant disease, but in this laboratory, as a result of cautious reporting, only about 60% are given "positive" or "suspicious" reports for malignant cells (Table 3). It is to be expected that a proportion

of the remaining 40% of serous effusions do not in fact contain any malignant cells, since they result from processes other than neoplastic involvement of serous membranes—for example, pulmonary collapse. On the other hand, there must be a number of specimens in which malignant cells are too few, or else not distinctive enough, to be recognisable on conventional cytological examination. Failure to detect these cells may have important implications in terms of patient management, and therefore any procedure which can reduce the threshold for their detection is to be welcomed, provided that the "false-positive" rate remains near to zero.

In the present study immunocytochemical staining with a small panel of monoclonal antibodies detected presumably malignant cells which had not previously been noticed in almost 30% (12 of 41) of cytologically "benign" serous effusions from patients with malignant disease. In addition 17% of samples gave immunocytochemical reactions which were suggestive of malignancy but insufficient *per se* to allow a confident diagnosis.

We cannot of course provide formal proof that the cells revealed by immunocytochemical staining were indeed malignant. We can only point to the rarity of false positives in our previous studies of unequivocally benign effusions: anti-CEA has given no such reactions in a series of 22 non-malignant samples (and this high specificity has recently been documented by Sehested *et al.*⁷) Ca 1 has only given two false positive results^{1,5} in 47 samples studied (combined data from two series). In this context it may be noted that we have not observed the high positivity rate for Ca 1 in benign mesothelium which was reported by Burnett *et al* in their immunocytochemical study of pleural biopsies,⁸ or by Pallesen *et al* using cells fixed in suspension before washing and sedimenting.⁹ Antibody HMFG-2 has proved less specific than anti-CEA and antibody Ca 1 for malignant cells but has often

Table 3 Cytological reports on pleural and peritoneal fluids 1965–76 inclusive

Cytological report	Final diagnosis				Total
	Malignant		Benign		
	Pleural	Peritoneal	Pleural	Peritoneal	
Positive	437	274	2	1	714
Suspicious	52	27	4	2	85
Negative	346	158	750	212	1466
Total	835	459	756	215	2265

"False-positive" = 0.4% of all positive reports.
 "False-suspicious" = 7.1% of all suspicious reports.
 "False-negative" = 38.9% of all cases of malignant disease.

Each entry represents a separate case, except where pleural and peritoneal effusions occurred together. "Final" diagnosis was obtained from review of hospital notes and death registrations; histological confirmation was not obtained in all cases.

provided corroborative evidence.

Additional evidence for the specificity of the reactions reported in the present study comes from the fact that in many cases the previously undetected cells revealed by immunocytochemical staining clearly possessed the morphological features of malignant cells. Obviously when issuing a clinical report of malignancy on the basis of immunocytochemical staining this criterion should be taken into account. Although not used in the present study there may also be a place for the consecutive staining technique described by To *et al*² in which the alkaline phosphatase reaction product is removed and the morphology of individual positive cells then assessed after conventional cytological staining.

The number of cases investigated in the present study is relatively small (41 patients) and a more extensive study is in progress. However, if the results obtained in this preliminary investigation are representative they suggest that the proportion of effusions from patients with cancer in which malignant cells can be detected should increase to almost 75%. This is a substantial improvement, and it may mean the avoidance of procedures which can be tiresome and painful and which are expensive to perform. Delay in obtaining a diagnosis also inevitably leads to delay in the initiation of the appropriate treatment.

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—for example, lymphoma; and it often gives 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.

We wish to acknowledge the help and advice of those listed in the following paper. Please refer to page 1163.

References

- ¹ Ghosh AK, Spriggs AI, Taylor-Papadimitriou J, Mason DY. Immunocytochemical staining of cells in pleural and peritoneal effusions with a panel of monoclonal antibodies. *J Clin Pathol* 1983;**36**:1154-1164.
- ² To A, Dearnaley DP, Ormerod MG, Canti G, Coleman DV. Epithelial membrane antigen: Its use in the cytodiagnosis of malignancy in serous effusions. *Am J Clin Pathol* 1982; **78**: 214-9.
- ³ Ashall F, Bramwell ME, Harris H. A new marker for human cancer cells. 1. The Ca antigen and the Ca 1 antibody. *Lancet* 1982;**ii**:1-6.
- ⁴ McGee JO'D, Woods JC, Ashall F, Bramwell ME, Harris H. A new marker for human cancer cells. 2. Immunohistochemical detection of the Ca antigen in human tissues with the Ca 1 antibody. *Lancet* 1982;**ii**:7-10.
- ⁵ Woods JC, Spriggs AI, Harris H, McGee JO'D. A new marker for human cancer cells. 3. Immunocytochemical detection of malignant cells in serous fluids with the Ca 1 antibody. *Lancet* 1982;**ii**:512-4.
- ⁶ Gatter KC, Abdulaziz Z, Beverley P *et al*. Use of monoclonal antibodies for the histopathological diagnosis of human malignancy. *J Clin Pathol* 1982;**35**:1253-67.
- ⁷ Sehested M, Ralfkjaer E, Rasmussen J. Immunoperoxidase demonstration of carcinoembryonic antigen in pleural and peritoneal effusions. *Acta Cytol* 1983;**27**:124-7.
- ⁸ Burnett RA, Deery ARS, Adamson MR *et al*. Evaluation of Ca 1 antibody in pleural biopsy material. *Lancet* 1983;**i**:1158.
- ⁹ Pallesen G, Jepsen FL, Hastrup J *et al*. Experience with the Oxford tumour marker (Ca 1) in serous fluids. *Lancet* 1983;**i**:1326.

Requests for reprints to: Dr AI Spriggs, Consultant Cytologist, Laboratory of Clinical Cytology, The Churchill Hospital, Headington, Oxford OX3 7LJ, England.