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Identification of second malignancies on effusions and fine-needle aspirates using a panel of monoclonal antibodies

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Summary The longer survival of neoplastic patients achieved through improvements of therapeutic regimens has increased the relative risk of developing a second primary tumour (SPT). In this context, conventional cytopathology can define tumour histotype only in a small fraction of cases. In this study, we have evaluated whether selected combinations of monoclonal antibodies (MAbs) to tumour-associated antigens (TAAs) can increase the accuracy of conventional morphology in detecting second primary tumours (SPTs) in two particularly difficult areas of cytodiagnosis, namely that of effusions and pulmonary fine-needle aspirates (FNAs). The immunocytochemical (ICC) analysis of 334 cytological specimens demonstrated that the use of our selected panel of MAbs could allow a more efficient identification of SPTs in comparison with conventional morphology. This diagnostic improvement was statistically significant ($P<0.0001$). The present findings show that the immunophenotyping of effusions and FNAs, providing a more accurate and objective identification of SPTs, may have significant therapeutic and epidemiological relevance.

Keywords: second neoplasias; cytological diagnosis; immunocytochemistry; metastases

Second primary tumours (SPTs) have been diagnosed more frequently in recent years during the clinical course of patients bearing haematopoietic (Abernathy et al, 1986; Tucker et al, 1988) and solid tumours (Lee, 1986; Kaldor et al, 1987). As early diagnosis of SPTs has major therapeutic and epidemiological relevance (Kaldor et al, 1987; Giardini et al, 1993), the development of new methods for the accurate detection of second malignancies should be attempted. In this context, exfoliative and FNA cytology may be considered a valuable and accurate diagnostic technique, easy to perform on a large scale during the follow-up of patients previously treated for malignant tumours. These methods also have the advantage of minimal morbidity and low cost (Koss, 1988). While this approach has been reported to be highly accurate in identifying metastatic cells, the possibility of defining the tumour histotype has been far less successful (Friedman et al, 1983; Hajdu et al, 1984). We have previously reported that the use of selected combinations of MAbs to TAAs can be applied to a number of areas of cytodiagnosis of solid tumours, thus increasing the accuracy of conventional morphology in identifying metastases from unknown primary tumour and in differentiating primary from metastatic lesions (Mottolese et al, 1993). In the present study, we have analysed, in a large group of patients with a past history of malignancy, whether the use of a similar panel of reagents may also be useful in detecting SPTs in two areas of cytopathology, which are particularly difficult on the basis of morphological criteria, namely that of effusions and of pulmonary FNA.

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MATERIALS AND METHODS

Patients

From January 1990 to June 1995, 334 cytological specimens, of which 91 were pulmonary FNA and 243 pleural and peritoneal effusions sampled from patients previously treated with chemo and/or radiotherapy for different malignant tumours, were analysed both cytologically and immunocytochemically (ICC). The series consisted of 93 patients with effusions and 52 with solitary or multiple pulmonary masses, which appeared within 5 years of a previous tumour, while 189 patients developed effusions (150 cases) or radiologically assessed lung lesions (39 cases) at least 5 years after the first tumour. Only those cases in which morphological examination assessed the presence of malignant cells on cytological specimens have been included in this study. Unsatisfactory or insufficient specimens were excluded.

Preparation of cell substrates

Pleural and peritoneal effusions were collected in sterile conditions using heparin (Liquemin Roche) as anticoagulant. Samples were centrifuged at 160 *g* for 10 min and the recovered cells were resuspended, after three washings with Hanks' balanced salt solution (Gibco Laboratories, Paisley, UK), at a density of 1×10^6 cells ml^{-1}. Red blood cells, when present, were removed by lysis with Tris/ammonium chloride pH 7.4, for 10 min at 37°C. Cytospins were obtained using a Shandon cytocentrifuge (Shandon, Runcorn, Cheshire, UK) and either stained according to the Papanicolau method for conventional morphological analysis or fixed in cold absolute acetone and immediately stored at –20°C for ICC evaluation. Pulmonary FNAs were performed under computerized tomography (CT) guidance with a 22-gauge needle placed on a 20-ml disposable syringe mounted

Table 1 Antigenic phenotype of different solid tumours identified with a panel of MAbs to TAAs[a]

MAbs										Antigenic Phenotype	
B72.3	B6.2	MBrl	MOv 18–19	OC-125	KS1/4	D612	RC38	PSA	Ep1–3		
+	+	+	–	–	NS[b]	–	–	–	–	Breast	carcinoma
+	–	–	+	+	NS[b]	–	–	–	–	Ovarian	carcinoma
+	–	–	–	+	+	–	–	–	–	Lung	carcinoma
+	–	–	–	–	–	+	–	–	–	Colon	carcinoma
+	–	–	–	–	–	–	+	–	–	Kidney	carcinoma
+	Ns[b]	–	–	–	NS[b]	–	–	+	–	Prostate	carcinoma
–	–	–	–	–	–	–	–	–	+	Melanoma	

[a]See Mottolese et al (1988, 1989, 1990, 1993, 1994). [b] NS, not significant to detect tumour origin.

Table 2 Immunocytochemical identification of SPTs in metastatic effusions from patients with a previous history of malignancy

First tumour	No. of patients	Immunocytochemical diagnosis[a]		
		Metastasis from first tumour	Metastasis from SPTs	Undefined malignant tumour
Breast carcinoma	95	74	8 Ovarian 3 Lung 2 Colon 1 Kidney 14	7
Ovarian carcinoma	61	52	2 Breast 1 Colon 3	6
Lung carcinoma	48	39	2 Melanoma	7
Colon carcinoma	17	15	1 Ovarian 1 Lung 2	
Melanoma	15	13	2 Lung	
Prostate carcinoma	7	6	1 Lung	
Total	243	199	24	20 (8.2%)

[a]Performed according to the pattern of reactivity summarized in Table 1.

on a special holder (Cameco 20 ml, Precision Dynamics, Burbank, CA, USA). Cellular specimens, smeared onto acid-clean glass slides, were fixed in 95% ethanol for conventional cytological diagnosis and in cold absolute acetone for ICC analysis.

Monoclonal antibodies and immunocytochemical assays

In order to identify the primary tumour site, we selected a large panel of MAbs directed to different TAAs. For the purpose of this study, we classified the reagents on the basis of their main tumour specificity, as follows.

MAbs against breast cancer-associated antigens

MAbs B72.3 and B6.2 were commercially obtained from Sorin Biomedica (Saluggia, Italy), while MAb MBrl was kindly provided by Professor MI Colnaghi (National Cancer Institute, Milan, Italy). MAb B72.3 identifies a high molecular weight glycoprotein expressed by the majority of adenocarcinomas and by 70% of breast carcinomas independent of histotype (Thor et al, 1986). MAb B6.2, which reacts with 80% of mammary adenocarcinomas, is also expressed by polymorpholeucocytes and by a high fraction of pulmonary and prostate carcinomas (Colcher et al, 1981). MAb MBrl recognizes a cell membrane neutral glycolipidic antigen, expressed by normal mammary epithelial cells, in about 70% of breast carcinomas and 40% of ovarian carcinomas (Canevari et al, 1983).

MAbs against ovarian cancer-associated antigens

MAbs MOv18 and MOv19 were obtained from Professor MI Colnaghi (National Cancer Institute, Milan, Italy) and MAb OC-125 from Cis Diagnostici (Tronzano Vercellese, Italy). The first two reagents display a highly restricted tumour specificity with 85% of serous and endometrioid ovarian carcinomas (Miotti et al, 1987), while the glycoprotein Ca-125, expressed by 80% of non-mucinous ovarian epithelial malignant tumours (Bast et al, 1991), is also present in 70% of non-small-cell lung carcinomas and in normal bronchial epithelium (Nouwen et al, 1986). In our study this antigen was detectable on activated mesothelial cells in only a small percentage of pleural and peritoneal effusions.

Table 3 Immunocytochemical identification of SPTs in pulmonary FNA of patients with a past history of malignancy

First tumour	No. of patients	Immunocytochemical diagnosis[a]			
		Metastasis from the first-tumour	Metastasis from a SPT	SPT[b]	Undefined
Breast carcinoma	30	25		3	2
Ovarian carcinoma	12	11	1[c]		
Colon carcinoma	6	4		2	
Melanoma	9	6		3	
Head and neck carcinoma	10	–	2[d]	2	6
Bladder carcinoma	3	1		2	
Kidney carcinoma	5	4		1	
NH lymphoma	16	12		4	
Total	91	63	3	17	8 (8.8%)

[a]Performed according to the pattern of reactivity summarized in Table 1. [b]Lung carcinoma. [c]Breast carcinoma. [d]One prostate carcinoma and one colon carcinoma. NH, non-Hodgkin's.

Table 4 Comparison between diagnostic potential of cytology and immunocytochemistry in identifying SPTs in effusions and pulmonary FNAs

First tumour	No. of patients	Metastasis		Second tumour		Undefined malignant tumour	
		Cytology	ICC	Cytology	ICC	Cytology	ICC
Breast carcinoma	125	97	99	7	17	21	9
Ovarian carcinoma	73	61	63	0	4	12	6
Lung carcinoma	48	37	39	0	2	11	7
Colon carcinoma	23	17	19	2	4	4	0
Melanoma	24	14	19	1	5	9	0
Prostate carcinoma	7	5	6	0	1	2	0
Head and neck carcinoma	10	1	0	0	4	9	6
NH lymphoma	16	11	12	3	4	2	0
Kidney carcinoma	5	4	4	0	1	1	0
Bladder carcinoma	3	1	1	1	2	1	0
Total	334	248	262[a]	14	44[a]	72 (21.5%)	28 (8.3%)

[a]The diagnostic improvement obtained through ICC analysis was statistically significant in comparison with standard cytology. $P<0.0001$ (McNemar's test). NH, non-Hodgkin's.

MAbs against lung, gastrointestinal, prostate and renal carcinomas

MAb KS1/4, directed to a lung cancer-associated antigen, kindly provided by Dr Reisfeld (Scripps Clinic, La Jolla, CA, USA), reacts with 95% of lung carcinomas, including small-cell lung cancer, and stains epithelial alveolar cells faintly and heterogeneously (Varky et al, 1984).

MAb D612 identifies 85% of primary and metastatic gastrointestinal carcinomas and was obtained from Dr J Schlom (NHI, Bethesda, USA) (Muraro et al, 1989). MAbs LI838 (PSA) (Dakopatts, Copenhagen, Denmark) and RC38 (Unipath Spa, Italy) demonstrate a highly restricted tumour specificity for prostatic (95%) and renal (97%) carcinomas respectively (Gallec et al, 1986, Oosterwijk et al, 1986).

MAbs against melanoma-associated antigens.

MAbs HMB45 (Gown et al, 1986) and Ep1–3 (Giacomini et al, 1987) were obtained from Dako and Immunology Laboratory, Regina Elena Cancer Institute, Rome, Italy respectively. MAb Ep1–3, which recognizes a high molecular weight melanoma-associated antigen (HMW-MAA), is made up by mixing equimolar concentrations of three reagents, Ep1, Ep2, Ep3. This pool of reagents demonstrates a restricted reactivity with both primary and metastatic melanoma, and also in amelanotic cells.

MAbs to intermediate filaments and lymphoid antigens

Additional ICC stainings were performed, in some instances, employing MAbs anti-paqcytokeratins (MNF116), anti-human leucocyte common antigen (CD45), anti-T cell (CD3 and CD45RO) and anti-B cell (CD45RA and CD20), purchased from Dako.

The pattern of reactivity of these reagents is described in Table 1. The use of multiple MAbs, as we previously demonstrated on a large number of different cytological specimens, is imposed by the lack of absolute tumour specificity of the selected reagents and by the need to overcome the heterogeneous expression of TAAs, which may often result in false-negative findings. (Mottolese et al, 1988, 1989, 1993, 1994).

The immunoreactivity of the MAbs was controlled repeatedly during the study using negative and positive control specimens. Cytological preparations were stained employing a sensitive biotin–streptavidin–immunoperoxidase method (LSAB Kit, Dako), and the enzymatic activity was developed with 3-amino-9-ethylcarbazole using Mayer's haematoxylin as nuclear counterstaining. Slides were mounted in aqueous mounting medium (Glycergel, Dako). The ICC findings were evaluated independently by two investigators who had no knowledge of the cytopathological diagnosis. The cytological and immunocytochemical findings were compared with those obtained by histopathology and/or with the clinical data obtained during patient follow-up.

Table 5 Comparison between ICC diagnoses of SPTs and clinical and pathological data obtained during the patient follow-up

First tumour	No. of patients	No. of SPTs ICC diagnosed	Confirmed diagnoses by clinical and pathological data
Breast carcinoma	125	17	15
Ovarian carcinoma	73	4	2
Lung carcinoma	48	2	1
Colon carcinoma	23	4	3
Melanoma	24	5	5
Prostate carcinoma	7	1	1
Head and neck carcinoma	10	4	4
Bladder carcinoma	3	2	2
NH lymphoma	16	4	4
Kidney carcinoma	5	1	0
Total	334	44	37[a]

[a]84% correlation with ICC diagnosis.

RESULTS

Immunocytochemical identification of SPTs in metastatic effusions from patients with a past history of malignancy

A total of 243 pleural or peritoneal effusions, which appeared in patients with a past history of malignancy, were analysed ICC using the panel of MAbs described in Table 1. This combination of reagents, in which both positive and negative immunoreactivities bear diagnostic information, identifies distinct antigenic phenotypes that can help to discriminate solid tumours of most common occurrence. As summarized in Table 2, 199 of the 243 effusions were defined, on the basis of their immunophenotype, as metastatic from the first tumour. In 24 patients, on the other hand, immunocytology suggested the presence of a metastasis from a SPT. Fourteen of these 24 patients had previously been treated for breast cancer, and the second malignancy originated in the ovary (eight cases), in the lung (three cases), in the colon (two cases) and in the kidney (one case). Three patients had a past history of ovarian carcinoma, two of whom developed a mammary and a colon carcinoma respectively. A melanoma appeared in two patients with a lung carcinoma, and a lung cancer in two

Table 6 Clinical and pathological confirmations of the SPTs immunocytochemically diagnosed

First tumour	Clinical presentation of disease	No. of cases	ICC diagnoses	Subsequent clinical evaluation	No. of confirmed ICC diagnoses	Histological diagnoses
Breast carcinoma	Pleural effusion	3	Lung cancer	Bronchoscopy + biopsy	2	1 Adc + 1 undifferentiated carcinoma
Breast carcinoma	Peritoneal effusion	8	Ovarian cancer	Laparoscopy + biopsy	7	5 Serous adc +2 undifferentiated carcinoma
Breast carcinoma	Peritoneal effusion	2	Colon cancer	Colonoscopy + biopsy	2	2 Undifferentiated adc
Breast carcinoma	Peritoneal effusion	1	Renal cancer	CT scan + biopsy	1	1 Renal cell carcinoma
Breast carcinoma	Pulmonary mass	3	Lung cancer	Bronchoscopy + biopsy	3	2 Adc + 1 undifferentiated carcinoma
		17			15	
Ovarian carcinoma	Pleural effusion	2	Breast cancer	Mammography + FNA	1	1 Ductal carcinoma
Ovarian carcinoma	Peritoneal effusion	1	Colon cancer	Colonoscopy + biopsy	1	1 Undifferentiated adc
Ovarian carcinoma	Pulmonary mass	1	Lung cancer	Bronchoscopy + biopsy	Not confirmed	Metastatic ovarian carcinoma
		4			2	
Lung carcinoma	Pleural effusion	2	Melanoma	Dermatological exam + surgery	1	1 Nodular melanoma (IV Clark lev)
Colon carcinoma	Pleural effusion	1	Lung cancer	Bronchoscopy + biopsy	1	1 Undifferentiated adc
Colon carcinoma	Peritoneal effusion	1	Ovarian cancer	Laparoscopy + biopsy	1	1 Serous adc
Colon carcinoma	Pulmonary mass	2	Lung cancer	Bronchoscopy + biopsy	1	1 Undifferentiated carcinoma
		4			3	
Melanoma	Pleural effusion	2	Lung cancer	Bronchoscopy + biopsy	2	2 Undifferentiated carcinoma
Melanoma	Pulmonary mass	3	Lung cancer	Bronchoscopy + biopsy	3	1 Adc + 2 undifferentiated carcinoma
		5			5	
Head and neck carcinoma	Pulmonary mass	2	Lung cancer	Bronchoscopy + biopsy	2	2 Undifferentiated carcinoma
Head and neck carcinoma	Pulmonary mass	1	Colon cancer	Colonoscopy + biopsy	1	1 Undifferentiated carcinoma
Head and neck carcinoma	Pulmonary mass	1	Prostate cancer	Transrectal ecotom. + biopsy	1	1 Adc
		4			4	
Prostate carcinoma	Pleural effusion	1	Lung cancer	Bronchoscopy + biopsy	1	1 Undifferentiated carcinoma
Bladder carcinoma	Pulmonary mass	2	Lung cancer	Bronchoscopy + biopsy	2	1 Adc + 1 undifferentiated carcinoma
Renal carcinoma	Pulmonary mass	1	Lung cancer	Bronchoscopy + biopsy	Not confirmed	Metastatic renal cell carcinoma
NHLymphoma	Pulmonary mass	4	Lung cancer	Bronchoscopy + biopsy	4	2 Undifferentiated adc + 2 undifferentiated carcinoma
Total		44			37	

NHL, non-Hodgkin's lymphoma. Adc, adenocarcinoma.

melanoma-bearing patients. An ovarian and a lung adenocarcinoma were ICC diagnosed in two patients who had been treated for a colon carcinoma, while in one patient with a prostate carcinoma, a lung cancer was diagnosed after 5 years from the previous tumour. ICC analysis was inconclusive in 20 out of 243 cases (82%). It is noteworthy that in effusions taken from patients 5 years after the previous neoplasia, the incidence of SPTs was similar to that observed in patients with a disease-free interval shorter than 5 years.

Immunocytochemical identification of SPTs in patients with pulmonary masses appearing within or after 5 years of a previous malignancy

A total of 91 patients affected by various non-pulmonary neoplasias were referred for percutaneous CT-FNA because of the radiographic appearance of a solitary (80) or multiple (11) pulmonary nodules of uncertain metastatic or primitive nature. As summarized in Table 3, in 63 out of 91 pulmonary FNAs the ICC diagnosis confirmed the metastatic origin of the pulmonary lesion, while in the remaining group of patients the immunocytological analysis identified three pulmonary metastases from a SPT coming from a new primary breast, prostate and colon carcinoma and 17 SPTs arising in the lung. In eight out of these 91 cases (8.8%), ICC assay was not able to indicate the tumour origin.

Comparison between cytological and ICC diagnosis

Table 4 compares the ICC and cytological diagnoses performed on the entire series of 334 specimens (243 effusions and 91 pulmonary FNAs) included in our study and described in detail in Tables 2 and 3. This analysis demonstrates that conventional morphology, although accurately recognizing the malignant nature of the cells, failed to differentiate between a metastasis from the previous tumour and a new SPT in 72 out of 334 (21.5%) specimens. All the 72 cases presented morphological features similar to the primary tumour. On the contrary, in 44 out of these 72 specimens cytologically undefined, ICC could identify 14 metastases from the first neoplasia and 30 SPTs. This diagnostic improvement was statistically significant ($P<0.0001$, McNemar's test). In the remaining 28 cases of uncertain primary or metastatic nature both morphologically and ICC, eight metastases from the previous neoplasia (four breast, two ovary, two head and neck carcinomas) and two SPTs arising in the lung of patients bearing a previous breast cancer were detected during the clinical course of the disease; the last 18 patients were lost to follow-up (FU).

The comparison between the ICC diagnosis of SPTs and the clinical or pathological data subsequently obtained is reported in Table 5. The use of the panel of MAbs on effusions and pulmonary FNAs allowed us to identify 44 SPTs out of 334 cases (13%), 37 of which have subsequently been confirmed with 84% correlation between ICC and clinical/pathological findings. In seven cases (one effusion from a lung, two from a breast, two from an ovarian carcinoma and two pulmonary FNAs sampled from patients with a previous colon and a renal carcinoma respectively), the presence of a SPT, suggested by the immunophenotyping, has never been clinically documented, therefore the neoplastic lesions were treated as metastases from the first tumour. Table 6 summarizes the ways in which the clinical and pathological confirmations of the 44 SPTs that ICC identified were made. The immunophenotyping in this series of patients allowed us to choose the clinical evaluation most suitable for obtaining additional tissue for the histopathological assessment. In all, 37 out of 44 SPTs have been confirmed, firstly, by clinical and imaging data and, subsequently, by histopathological diagnosis.

DISCUSSION

While SPTs have for a long time been incidental autopsy findings, an increasing number of these tumours are currently diagnosed during the follow-up of patients treated for cancer with an incidence ranging from 5% to 30% (Friedman et al, 1983; Cahan, 1977). The causes of the development of SPTs may include genetic, hormonal, environmental and treatment-related factors (Cooper et al, 1989). Furthermore, longer survival times, owing to improved therapeutic results, increase the relative risk of a new tumour unrelated to the first (Cahan, 1977). Cytopathology is recognized, at present, as a valuable diagnostic technique in almost any tumour type; thus, efforts to apply this methodology to the identification of SPTs are currently ongoing (Golub and Lefemine, 1969; Friedman et al, 1983; Giardini et al, 1993).

A diagnosis of SPT is entertained when, according to the traditional criteria of Warren and Gates (1932), (1) the cytological findings are compatible with a malignant process; and (2) clear morphological differences are observed between the examined specimens and the expected cytology. Nevertheless, in a number of cases SPTs may present morphological features so similar to the first tumour that cytological methods are unable to distinguish a metastasis from a second neoplasia. The most critical differential diagnosis was between a metastatic and a second primary adenocarcinoma, as in the case in which a SPT is subsequent to a lung, mammary, ovarian or colon adenocarcinoma. In addition, the differential diagnosis between squamous cell carcinomas and adenocarcinomas may not be unequivocal in poorly differentiated tumours (Giardini et al, 1993; Van der Gaast et al, 1996). Also, in lymphoproliferative diseases, the cytological diagnosis is easily feasible only when monomorphic cellularity occurred, whereas the assessment of a diffuse non-Hodgkin's disease with a polymorphic cell population is more difficult (Pilotti et al, 1993). A partial success in overcoming these limitations has been achieved by combined morphology with immunophenotyping. This has so far mainly relied on the use of MAbs to intermediate filaments and to other markers of relatively low specificity. Therefore, a significant number of cases cannot be diagnosed beyond the general term of 'adenocarcinoma' and 'carcinoma' until the histological examination becomes available (Giardini et al, 1993; Van der Gaast et al, 1996). Because previous studies of ours have shown that the combined evaluation of a large and well-characterized panel of MAbs on cytological specimens has the capability of detecting tumour origin in patients with cryptic tumours (Mottolese et al, 1988, 1989, 1990, 1994), we have routinely used this selected combination of reagents to establish more critically the diagnostic accuracy of the method in identifying otherwise undiagnosed SPTs. The results of this study, which reports our experience over the last 5 years, clearly demonstrate that this diagnostic approach offers an unprecedented accuracy in detecting SPTs, mainly in those lesions clearly malignant on the basis of cytological features, in which an undifferentiated morphology cannot permit establishment of their primary or metastatic nature.

This is supported by two types of evidence. Firstly, our immunocytodiagnosis has been consistently confirmed during the patient follow-up by clinical and/or pathological data in a high

percentage of cases; secondly, the type of SPTs identified reflects, in most instances, known epidemiological associations among neoplasias. As already reported (Lee et al, 1986), we have in fact found that in breast cancer patients, ovarian carcinoma is the most frequent SPT irrespective of the disease-free interval. Along the same lines, in 61 effusions, which appeared in patients with a previous history of ovarian carcinoma, we could identify one metastasis from colon and two from breast carcinomas, which are the most common SPTs in patients bearing this female genital tract neoplasia (Kaldor et al, 1987). As reported by Abernathy et al (1986), the occurrence of a single pulmonary mass in patients with a previously diagnosed carcinoma or haematopoietic tumour must always be accurately evaluated with the aim of establishing whether the lesion is a metastasis from the first neoplasm or a second primary lung cancer. Our study has indeed shown that in the group of patients bearing pulmonary nodules, which appeared after chemo and/or radiotherapy, a second malignancy was detected in 20 out of 91 cases. Seventeen had a SPT in the lung, while in three cases the lesion was identified by ICC and subsequently clinically and/or pathologically as metastatic from a colon, a breast and a prostate carcinoma. These findings bear particular relevance in melanoma patients who have an increased risk of developing a pulmonary SPT (Perry et al, 1986), and in patients with head and neck cancer, which most frequently metastatize to the lung (Cooper et al, 1989; McDonald et al, 1989). In addition, metastatic melanoma may often be amelanotic, as also evident in our group of patients, thus raising further diagnostic problems. In fact, in four of 15 effusions from patients with a past history of melanoma and in two of 48 effusions from patients with a lung cancer, cytology failed to recognize melanoma metastatic cells owing to the lack of pigmentation. In this context, ICC may have therapeutic implications of particular clinical relevance, since Hainsworth et al (1991) demonstrated that patients with undifferentiated tumours, diagnosed as metastatic melanoma on the basis of ICC criteria, may benefit significantly from a cisplatin-based regimen of chemotherapy.

In the present study, the comparison with standard diagnosis showed that ICC was more efficient than cytology in identifying a tumour coming from a specific second primary tumour rather than from the original neoplasia. When this methodological approach was compared statistically with conventional cytology, employing McNemar's test, a significant ($P<0.0001$) diagnostic improvement could be observed. In conclusion, our results indicate that the immunophenotyping of cytological specimens, allowing more accurate detection of a second neoplasia, could provide the pathologist and the epidemiologist with a molecular survey of these malignancies and the clinicians with guidelines for correct prognostic evaluations and therapeutic planning.

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