Diagnosis of Major Tumor Categories in Fine-Needle Aspirates Is More Accurate When Light Microscopy is Combined With Intermediate Filament Typing

A Study of 403 Cases

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Intermediate filament (IF) typing of tumor cells with monoclonal antibodies was applied to 403 fine-needle aspirates. In 271 cases specific cytologic diagnosis of tumor type was apparent from clinical data and light microscopic study alone. Intermediate filament typing confirmed the tumor type in 262 cases and changed an erroneous cytologic diagnosis of major tumor type in nine cases. In a second group of 132 difficult cases, where the tumor type could not be revealed with certainty, IF typing confirmed the cytologic suggestion of tumor type in 50 cases, changed it in nine cases, and helped resolve ambiguities in cytologic diagnosis in 59 cases. It did not help in 14 cases. Thus IF typing adds independent objective differentiation specific information to descriptive tumor typing currently used in aspiration cytologic study. When combined with the morphologic analysis of tumor cells and clinical information it can refine the cytologic diagnosis of major tumor types and prevent error.

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FINE-NEEDLE ASPIRATION BIOPSY has become widely recognized as a method of obtaining a morphologic diagnosis of primary and metastatic tumors. In expert hands, it has proved quite accurate in distinguishing benign from malignant lesions. The specific diagnosis of major tumor types such as carcinoma, sarcoma, malignant lymphoma, or malignant melanoma can be easily made if morphologic signs of differentiation can be distinguished in the tumor cells.[1–5] However, especially in metastatic lesions, the accurate cytologic diagnosis can be very difficult if perceptible signs of differentiation of tumor cells at the light microscopic level are absent. This accounts for poor interobserver reproducibility of the cytologic diagnosis of tumor type as compared with excellent reproducibility in cases having tumor cells with obvious light microscopic features of differentiation.[5–8]

On the other hand, in aspiration cytologic examination, accuracy of cytologic diagnosis of tumor type depends critically on the availability to the cytopathologist of reliable clinical, radiologic, and other information and not only on the morphologic features of cells in smears. As pointed out by Koss, "The same diagnostic parameters when applied to different organs will not work and may, in fact, result in diagnostic disasters."[9] Since the diagnosis of a particular tumor type often has important therapeutic and prognostic consequences, additional differentiation-specific or tissue-specific markers are needed in fine-needle aspirates (FNA) to provide information that combined with analysis of morphologic features of tumor cells and available clinical data can lead to precise diagnosis of tumor type.

In recent years the diagnostic value of intermediate filaments—as a group of such markers—has been documented in histologic sections of a wide variety of human tissues and tumors.[10–14] A number of recent studies have suggested that intermediate filament (IF) typing of tumor cells can be useful in differential diagnosis of FNA.[5,7,15–18] Here we report the results of the routine use of a panel of monoclonal antibodies to intermediate filaments on FNA in two cytopathology laboratories over a period of 2 years and its influence on the diagnostic accuracy in assessment of major tumor categories.

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Materials and Methods

Alcohol-fixed FNA biopsy specimens of 403 malignant tumors were collected over a period of 2 years in two cytopathology laboratories. They belonged to two groups: group A where specific cytologic diagnosis of tumor type was provided (271 cases collected at random) and group B in which diagnosis of major tumor categories could not be made with confidence (132 cases). Altogether there were 133 primary, 185 metastatic, 14 recurrent tumors, and 71 non-Hodgkin's malignant lymphomas (ML). Nonpalpable tumors underwent aspiration under computer tomography or ultrasonography guidance.

The aspirates used in this study are listed in Tables 1 to 7. From each aspirate, at least four cytologic smears were prepared on glass slides. Two smears were fixed within a few seconds in 100% ethanol precooled to 4°C. After 1 to 6 hours at 4°C, they were air dried and then stored at −20°C to −30°C for 1 to 30 days before staining. Occasionally smears stored for several months at −30°C or −70°C were used. The other two smears were fixed, again within a few seconds, in 100% ethanol for 30 minutes and subsequently stained with hematoxylin and eosin for light microscopic study.

Immunohistochemical Procedures

For immunofluorescence, the smears were further fixed in 100% acetone at −10°C for 2 minutes. After a wash with phosphate-buffered saline (PBS), 10 μl of the first antibody was added, usually as a hybridoma supernatant, and the smears were incubated for 45 minutes at 37°C in a humid chamber. Often, because of the restricted amount of material available, circles were made on the glass slide and parallel incubations with different first antibodies were performed on different parts of the same glass slide. After washing well with PBS, 10 μl of fluorescein-labeled goat anti-mouse IgG (Cappell Laboratories, Cochraneville, PA), diluted 1:40, was added, and the smears were incubated for a further 30 minutes at 37°C. After an additional wash with PBS, the smears were mounted in Mowiol 4-88 (Hoechst, Frankfurt, FRG) and examined in the fluorescence microscope.

Double-label fluorescence staining was performed when necessary to adequately interpret the results.[18] Usually a mouse monoclonal keratin (K) antibody and a guinea pig vimentin (V) antibody were given simultaneously as first antibodies. After washing in PBS, the two second antibodies were given simultaneously. Appropriate filters were used to separate the fluorescein isothiocyanate (FITC) and rhodamine labels in the fluorescence microscope.

Monoclonal antibodies used in this study were as follows: (1) KL1 a broad-specificity keratin antibody[19] (Dianova, Hamburg, FRG); (2) lu-5, a broad-specificity keratin antibody[20] (obtained from Dr. C. Staehli, Central Research Division, F. Hoffmann-La Roche and Co., Ltd., Basel, Switzerland); (3) CK-2, a keratin antibody specific for keratin 18, which is typical of simple and transitional epithelia[21]; (4) V-9, a monoclonal vimentin antibody[22]; (5) DE-B-5, a monoclonal desmin (D) antibody[23]; (6) NR-4, an antibody specific for the 68-kilodalton polypeptide of mammalian neurofilaments (NF).[24] § It should be noted that hybridoma supernatants have been used in the current studies, whereas many of the same antibodies that are commercially available are sold as ascites fluids. Therefore tests to establish appropriate working dilutions are necessary. Although most results in this report have used immunofluorescence methods satisfactory results can be obtained with immunoperoxidase or alkaline phosphatase detection methods. Again, however, assays to find the appropriate working dilution of the first antibodies are necessary.

Results

This study is based on IF typing of tumor cells in FNA biopsy specimens of 403 malignant tumors for which adequate follow-up information was available and in which the tumors were also in large majority subsequently characterized by histologic analysis. The fine-needle aspirates were prepared over a period of 2 years in two cytologic laboratories and represent approximately 8% of the specimens examined by fine-needle aspiration in conventional cytologic study over this time period. Fifty-three carcinomas, 14 malignant lymphomas, 14 sarcomas (including one epithelioid sarcoma and one synovial sarcoma), seven malignant melanomas, eight Wilms' tumors, one Triton tumor, and one carcinoid of the lung have been published previously in references.[7,17,18,25–27]

Fine-needle aspirates were divided for this study in two groups. Group A contained 271 cases collected at random in which the cytologic diagnosis of tumor type appeared unambiguous and where an unambiguous diagnosis of tumor type, *e.g.*, carcinoma (Ca), sarcoma (Sa), malignant lymphoma (ML), or malignant melanoma (MM) based only on the conventional light microscopic appearance could be made. Intermediate filament typing of tumor cells provided further independent evidence for the tumor type thus confirming the cytologic diagnosis in 262 of 271 of these cases. This group served to further correlate results obtained by conventional cytologic study and by IF typing (Tables 1 and 2). In nine cases the initial cytologic diagnosis was changed after IF typing (Table 3).

Group B contained 132 cases and included the majority

§ CK2, V-9, DE-B-5 (or DE-R-11, a second desmin antibody), and NR4 can be purchased from Amersham (Little Chalfont, England); Biomakor (Rehovot, Israel); Boehringer Mannheim (FRG); Dako (Klostrup, Denmark); ICN Immuno Biologicals (Lisle, IL), or Oncogene Science (Manhasset, NY).

TABLE 1. Cytologic Diagnosis of Tumor Type Confirmed by Intermediate Filament Typing of Tumor Cells in Fine-Needle Aspirates (Group A)

Cytologic diagnosis	No. of cases	Histologic diagnosis	IF typing with MAb to V	K	D	NF
ML	24	ML	24/24	0/20		
MM	10	MM	10/10	0/6		
Sa	20	Sa-osteo (4), Sa-mfh (2), Sa-lipo (1), Sa-chondro (1), Sa-nonmuscle (12)	20/20	0/3	0/3	
	4	Sa-synovial	4/4	4/4		
	1	Sa-epithelioid	1/1	1/1		
	4	Sa-leiomyo	4/4		4/4	
Sa-mfh	2	Sa-mfh	2/2			
Sa-lipo	1	Sa-lipo	1/1			
Sa-synovial	1	Sa-synovial	1/1	1/1		
Sa-leiomyo	2	Sa-leiomyo	2/2		2/2	
Plasmacytoma	1	Plasmacytoma	1/1	0/1		
Histiocytosis X	2	Histiocytosis X	2/2	0/2		
Carcinoid (lung)	1	Carcinoid	0/1	1/1		1/1
	73					

V: vimentin; K: keratin, D: desmin, NF: neurofilaments; IF: intermediate filaments; ML: malignant lymphoma; MM: malignant melanoma; Sa = sarcoma; mfh: malignant fibrous histiocytoma; MAb: monoclonal antibodies.

Notice that in each case the results of IF typing are consistent with the cytologic and histologic diagnoses. ML: 19 in lymph nodes, five in soft tissues; MM: eight lymph node metastases, one liver metastasis, one skin recurrence; Sa: 25 primary tumors, five recurrent, five metastatic.

of the cases encountered in the 2-year period in which malignant cells were easily diagnosed cytologically but where the tumor type could not be revealed with certainty (2.6% of the total). Usually, however, on the basis of light microscopic analysis of morphologic features of the tumor cells and the available clinical data one or two suggestions as to the possible major tumor type were given. Intermediate filament typing of tumor cells of cases in group B confirmed, changed or refined the initial cytologic suggestion of tumor type and in most of these instances made the final diagnosis more specific and accurate (Tables 4–6).

Group A

Cytologic diagnosis of tumor type confirmed: In 262/271 cases in group A (96.7%) where the diagnosis of tumor type from conventional cytologic study appeared unambiguous, this was confirmed by the results of IF typing of tumor cells in aspirates (Tables 1 and 2). They included 24 malignant lymphomas (20 V+K−; four V+, K not tested), ten malignant melanomas (Figs. 1A and 1B) (all V+, six K− with the rest not tested), one plasmacytoma, and two cases of histiocytosis X (V+K−), and one carcinoid in the lung (K+NF+V−).

All 35 sarcomas were vimentin positive. The tumor cells in five synovial sarcomas and one epithelioid sarcoma coexpressed vimentin and keratin. Six leiomyosarcomas coexpressed vimentin and desmin.

Tumor cells of the 189 carcinomas listed in Table 2 expressed keratin as assayed with a broad specificity keratin antibody irrespective of whether they were of primary or metastatic origin. Most carcinomas listed in Table 2 are keratin positive, vimentin negative (Figs. 2A–2C). However vimentin was coexpressed in the tumor cells of all primary or metastatic thyroid and renal cell carcinomas in our series (Figs. 3A–3C). Vimentin coexpression also was noted in single examples of primary and metastatic small cell lung carcinoma and of primary adrenocortical carcinoma as well as in two recurrent endometrial carcinomas. In addition vimentin coexpression was noted in five of eight ovarian carcinomas, two of 13 primary breast carcinomas, one of three metastatic carcinomas of the prostate, one metastatic squamous carcinoma of the larynx, and seven metastatic carcinomas where the primaries were unknown.

Cytologic diagnosis of tumor type changed: In nine of 271 cases in group A (3.3%), the original cytologic diagnosis was carcinoma (Table 3). However, IF typing showed only vimentin and not keratin in tumor cells in these nine cases. In seven cases numerous, quite pleomorphic small anaplastic tumor cells with scanty or moderate amounts of cytoplasm were present dispersed in the smears. Some were arranged in small loosely cohesive groups. After IF typing and reconsideration of the cytologic data these cases were finally diagnosed as malignant lymphomas, two of which involved breast lesions and five lymph nodes (Figs. 4A–4D). Subsequent examination of biopsy specimens in histologic analysis revealed large cell malignant lymphomas in all seven cases.

Case 310 was initially diagnosed cytologically as metastatic carcinoma. Tumor cells had epithelial-type morphologic features and variable amounts of cytoplasm. They were found dispersed and arranged in clusters of

variable size. Tumor cell nuclei had large nucleoli. A few binucleated cells were found as well as some intranuclear cytoplasmic inclusions. There was no melanin pigment. However, since the tumor cells were found to be vimentin positive and keratin negative, the morphologic features of the cells were reevaluated and a diagnosis of amelanotic malignant melanoma provided. This was later confirmed by histologic examination. The morphologic features of the tumor cells in Case 336 fitted neither a diagnosis of malignant melanoma nor of sarcoma. Since the cells were only vimentin positive the diagnosis of carotid body tumor was suggested, consistent with the location of this lymph node-like cervical nodule. This suggestion was confirmed by arteriography and further supported by heavy bleeding during the subsequent operation. Due to the bleeding the nodule could not be removed for histologic examination.

Group B

Cytologic suggestion of tumor type confirmed: In 50 cases the cytologic suggestion of tumor type was confirmed by IF typing (Table 4). These cases included 14 sarcomas, all of which were vimentin positive. Desmin positivity of the tumor cells in four cases confirmed the cytologic suggestions of rhabdomyosarcoma and leiomyosarcoma. One tumor which contained a mixture of vimentin and desmin and neurofilament-positive tumor cells was diagnosed as Triton tumor.

The cytologic suggestion of carcinoma was confirmed in 13 cases, since all contained keratin-positive tumor cells. In addition, true coexpression of keratin and vimentin was found in two carcinomas of the kidney, two large cell type carcinomas of the thyroid, and one undifferentiated carcinoma of the lung. True coexpression of keratin and neurofilaments was found in three cases of Merkel cell carcinoma. The presence of vimentin in tumor cells and the absence of keratin helped to confirm the cytologic suggestion of malignant melanoma in nine cases, in which no melanin pigment was present in tumor cells and malignant lymphoma in eight cases. Tumor cells from two neuroblastomas were neurofilament positive and vimentin negative. Finally, IF typing of four malignant tumors in which cytologic study suggested Wilm's tumor revealed tumor cells containing vimentin and/or keratin as well as in two cases an additional population of desmin-positive cells. Thus the diagnosis of Wilms' tumor was confirmed by IF typing.

Cytologic suggestion of tumor type changed: There were nine difficult aspirates in this group. All except one were composed of small, anaplastic malignant tumor cells, which displayed no apparent signs of differentiation at the light microscopic level (Table 5). Therefore, although they were easily diagnosed as malignant, the possible tumor type could not be decided with confidence. In three instances malignant lymphoma was suggested, but since the tumor cells were vimentin negative and keratin positive, the diagnosis of malignant lymphoma had to be reevaluated. In two cases, in which antibodies against keratins and neurofilaments revealed distinct IF aggregates (IF bodies) usually in a perinuclear position, a final diagnosis of Merkel cell carcinoma was provided.[25,28] A further case was diagnosed as anaplastic carcinoma.

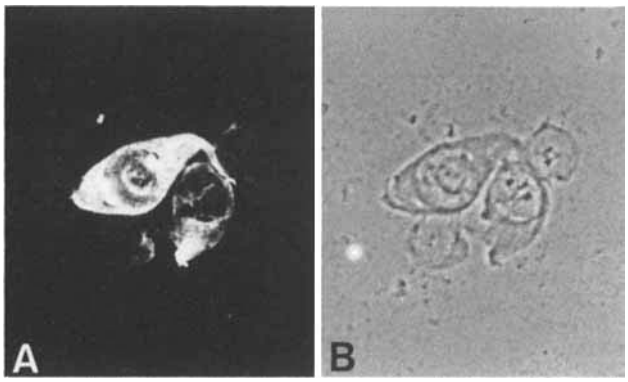
In five cases, carcinoma, small cell anaplastic carcinoma, transitional carcinoma (lymphoepithelioma), and carcinoid were suggested from the cytologic examination.

TABLE 2. Cytologic Diagnosis of Carcinoma Confirmed by Intermediate Filament Typing of Tumor Cells in Fine-Needle Aspirates (Group A)

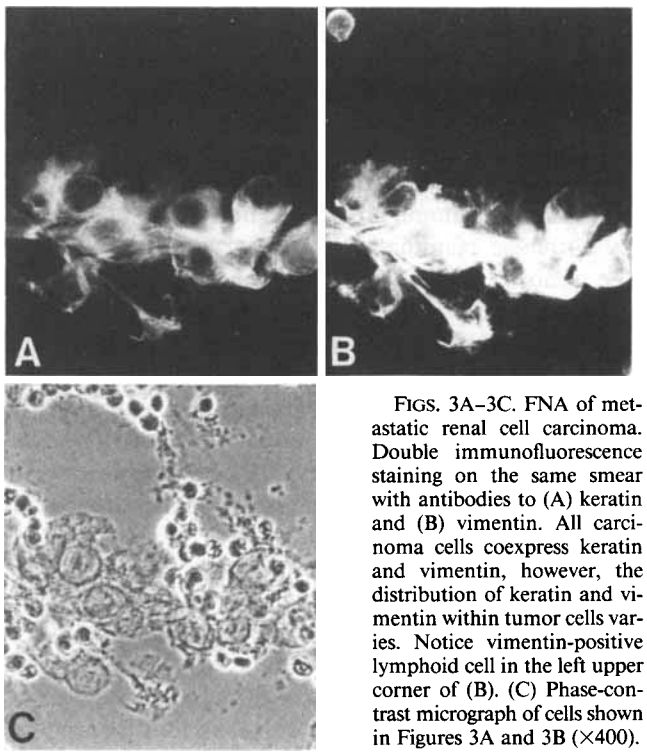
Origin of carcinoma	No. of cases			IF typing with MAb to		
	P	M	R	K	V	NF
Ovary	5	3		8/8	5/8	
Thyroid						
Papillary	4	4		8/8	8/8	
Follicular		2		2/2	2/2	
Large cell	1			1/1	1/1	
Medullary	1			1/1	1/1	1/1
Kidney	4	13		17/17	17/17	
Lung						
SC	1			1/1	0/1	
AdC	1	1		2/2	0/2	
AnC-lc	1			1/1	0/1	
AnC-sc	3	9		12/12	2/12	
Breast	16	7		23/23	2/13	
Endometrium			2	2/2	2/2	
Adrenal gland	1			1/1	1/1	
Prostate	2	1		3/3	1/3	
Stomach		3		3/3		
Pancreas	9			9/9	0/9	
Large bowel	3	3		6/6	0/6	
Liver-cell	5			5/5	0/5	
Squamous Ca						
Skin	3	4		7/7	0/3	
Larynx		4		4/4	1/2	
Nasopharynx	1			1/1	0/1	
Tonsil	1			1/1	0/1	
Uterus–cervix		5		5/5	0/2	
Esophagus		1		1/1	0/1	
Tongue		1		1/1	0/1	
Vulva		1		1/1	0/1	
Primary not known						
AnC		4		4/4	1/4	
SC		8		8/8	0/5	
AdC		31		31/31	3/22	
Ca		20		20/20	3/15	
	62	125	2			

P: primary; M: metastatic; R: recurrent; K: keratin; V: vimentin; NF: neurofilaments; SC: squamous Ca; Ca: carcinoma; AdC: adenocarcinoma; AnC: anaplastic carcinoma; lc: large cell; sc: small cell; MAb: monoclonal antibodies.

All cases were later confirmed histologically except for nine pancreatic carcinomas, one liver cell carcinoma, five AdCa, and nine Ca with unknown primary. In these cases histologic types could not be obtained; however, follow-up was consistent with the diagnosis of carcinoma. In each case the finding of keratin positivity is consistent with the diagnosis of carcinoma. Some carcinomas coexpress keratin and vimentin (see text).



FIGS. 1A AND 1B. FNA of malignant melanoma metastatic to the lymph node. (A) Tumor cells labeled with the V9 vimentin antibody. Notice strong staining of the cytoplasm of tumor cells (indirect immunofluorescence). (B) Phase-contrast picture of cells shown in Figure 1E (×400).

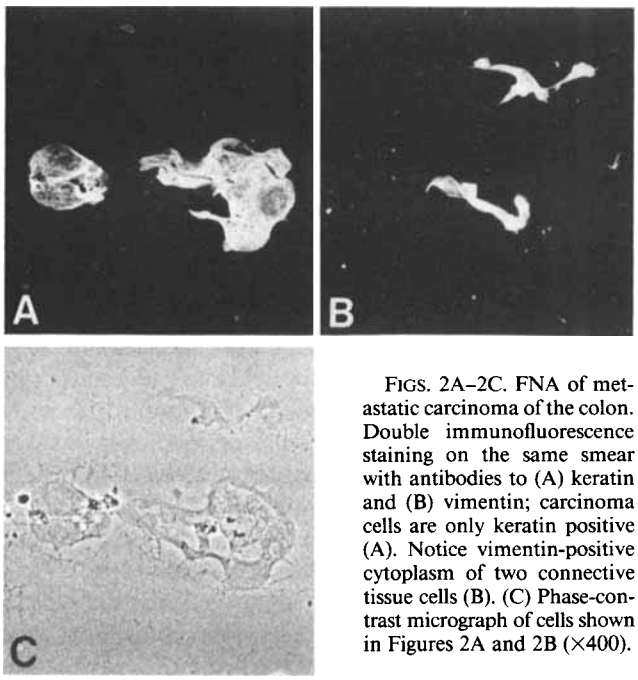


FIGS. 3A–3C. FNA of metastatic renal cell carcinoma. Double immunofluorescence staining on the same smear with antibodies to (A) keratin and (B) vimentin. All carcinoma cells coexpress keratin and vimentin, however, the distribution of keratin and vimentin within tumor cells varies. Notice vimentin-positive lymphoid cell in the left upper corner of (B). (C) Phase-contrast micrograph of cells shown in Figures 3A and 3B (×400).

Since tumor cells were only vimentin positive and keratin negative, four of them were finally diagnosed as malignant lymphomas and one as amelanotic malignant melanoma.

Ambiguity of cytologic diagnosis of tumor type resolved: The distinction between carcinoma and malignant lymphoma could not be made cytologically in 29 cases (Table 6). When the results of IF typing were known, 22 cases were diagnosed as malignant lymphoma (V+K−), six as carcinomas (V−K+) and one as malignant melanoma (V+K−).

There were nine cases in which the question of carcinoma or sarcoma could not be answered unequivocally at the light microscopic level. After IF typing seven were diagnosed as sarcomas (V+K−), including one leiomyosarcoma (V+D+), and two as carcinomas: one Merkel cell carcinoma of the skin (K+NF+V−) and one metastatic carcinoma where the primary was unknown.



FIGS. 2A–2C. FNA of metastatic carcinoma of the colon. Double immunofluorescence staining on the same smear with antibodies to (A) keratin and (B) vimentin; carcinoma cells are only keratin positive (A). Notice vimentin-positive cytoplasm of two connective tissue cells (B). (C) Phase-contrast micrograph of cells shown in Figures 2A and 2B (×400).

There were five cases in which the differential diagnostic question of carcinoma or malignant melanoma could not be definitely solved at the light microscopic level. In three malignant melanomas tumor cells were vimentin positive, keratin negative but contain no melanin pigment. The remaining two cases were diagnosed as carcinomas since they were keratin positive. One of them, a kidney tumor, showed true coexpression of vimentin in tumor cells indicating a renal cell carcinoma.

The presence of vimentin and the absence of keratin helped in making the diagnosis of plasmacytoma in one of two cases in which the distinction between carcinoma and plasmocytoma could not be made on the cytologic evidence alone. In the other case carcinoma was diagnosed (V−K+).

Malignant lymphoma (V+K−D−NF−) was finally diagnosed in three of four cases in which a differential diagnosis between malignant lymphoma and sarcoma, neuroblastoma or hepatoblastoma had to be made. Tumor cells in the remaining case coexpressed vimentin and desmin indicating leiomyosarcoma.

In two cases cytologic analysis was in favor of either Wilms' tumor or rhabdomyosarcoma. In one of them

TABLE 3. Cytologic Diagnosis of Tumor Type Changed as a Result of Intermediate Filament Typing of Tumor Cells in Fine-Needle Aspirates (Group A)

Case	Age	Site	Initial cytologic diagnosis	IF typing with MAb to V	IF typing with MAb to K	Definitive cytologic diagnosis (LM + IF)	Histologic diagnosis
240	75	Breast	AnC-sc	+	−	ML*	ML
49	55	Breast	AnC-sc	+	−	ML	ML
241	75	LNa	AnC-sc	+	−	ML*	ML
56	55	LNc	AnC-sc	+	−	ML	ML
262	50	LNc	AnC	+	−	ML*	ML
12/A	78	LNc	AnC	+	−	ML	ML
263	17	LNi	Ca	+	−	ML*	ML
336	33	Neck	Ca	+	−	Carotid body tumor†	
310	76	LNa	Ca	+	−	MM	MM

MAb: monoclonal antibodies; V: vimentin; K: keratin; LM: light microscopic study; IF: intermediate filaments; LN: lymph node; a: axillary; c: cervical; i: inguinal; AnC: anaplastic carcinoma; sc: small cell; Ca: carcinoma; ML: malignant lymphoma; MM: malignant melanoma; LCA: leucocyte common antigen; FNA: fine-needle aspirates.

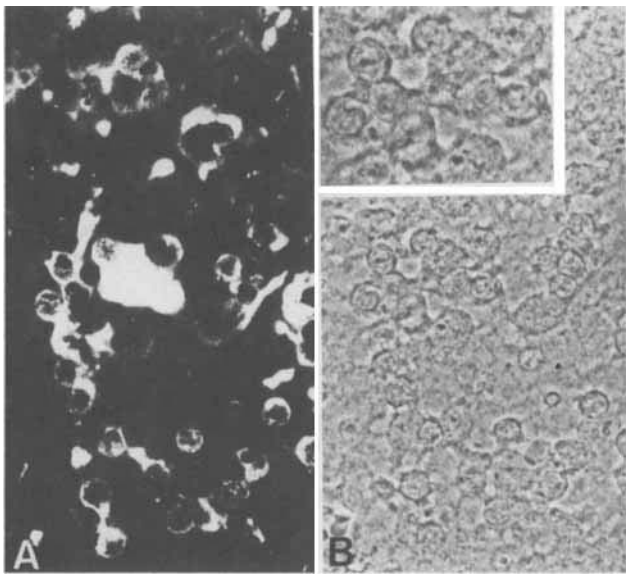
* Tumor cells in FNA also LCA positive.

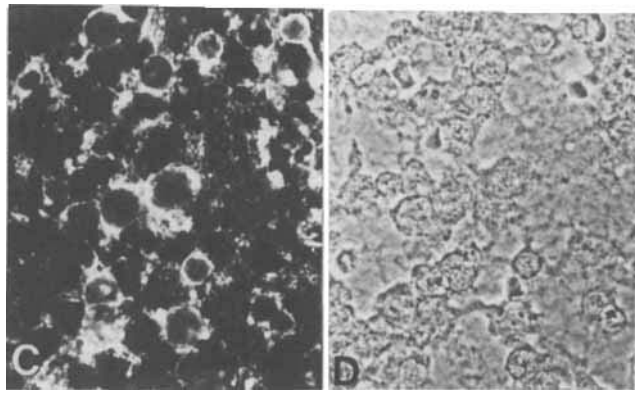
† Histologic typing not done (see text); finding of V+K-tumor cells excludes a diagnosis of carcinoma.

Wilms' tumor was diagnosed (K+V+ some D+ cells); in the other, nonmuscle sarcoma (V+K−D−NF−). In one case Ewing's tumor was finally diagnosed (V+D−NF−) and not a neuroblastoma which was also taken into consideration in the cytologic diagnosis.

In seven cases only malignant cells were diagnosed in the smear with no further suggestion being made. Six of them were malignant small round cell tumors of childhood. Intermediate filament typing helped to diagnose three of them as Wilms' tumors, one as neuroblastoma, and two as sarcomas including one rhabdomyosarcoma (Figs. 5A and 5B). The remaining case was diagnosed as adenocarcinoma.

Cytologic difficulty in diagnosis of tumor type not resolved: There were 14 cases in which IF typing of tumor cells did not help in further diagnosis of the tumor type, although the results of IF typing are consistent with the cytologic diagnosis (Table 7). One metastatic amelanotic melanoma could not be unambiguously diagnosed, since the differential diagnosis included sarcoma and both tumors are vimentin positive. For the same reason one malignant lymphoma appearing as a soft tissue tumor could not be distinguished from primary soft tissue sarcoma. However, use of leukocyte common antigen, which was positive on the tumor cells, allowed a diagnosis of lymphoma. In two cases, one neuroblastoma and one adrenal cortical carcinoma, no IF were found in tumor cells. In one retroperitoneal undifferentiated sarcoma in a child the cytologic diagnosis was malignant small round blue cell tumor and therefore several tumor types had to be taken into consideration including malignant lymphoma. Since the tumor cells were only vimentin positive, neu-





FIGS. 4A–4D. FNA of malignant lymphoma (breast). (A) Tumor cells labeled with the V9 vimentin antibody (indirect immunofluorescence). (B) Phase-contrast picture of tumor cells shown in Figure 4A. Notice prominent nucleoli (*Inset:* ×500). (C) Tumor cells labeled with antibody to LCA (indirect immunofluorescence). (D) Phase-contrast picture of the cells shown in Figure 4C (×250).

TABLE 4. Cytologic Suggestion of Tumor Type Confirmed as a Result of Intermediate Filament Typing of Tumor Cells in Fine-Needle Aspirates (Group B)

Initial cytologic diagnosis	No. of cases			IF typing with MAb to				Definitive cytologic diagnosis*
	P	M	R	V	K	D	NF	
m-Sa?	6		1	7/7	0/5	0/4		Sa-nonmuscle
	1			1/1		1/1	1/1	Triton tumor
m-Sa-rhabdo?	2			2/2		0/2		Sa-nonmuscle
	1	1		2/2	0/2	2/2		Sa-rhabdo
m-Sa-leiomyo?	2			2/2		2/2		Sa-leiomyo
m-Ca?		5		0/4	5/5			Ca†
	2	2	1	5/5	5/5			Ca‡
m-Ca-Merkel?		1	2	0/3	3/3		3/3	Ca-Merkel
m-MM?		9		9/9	0/9			MM§
M-ML?	8			8/8	0/8			ML
m-Neuroblastoma?	2			0/2			2/2	Neuroblastoma
m-Wilms?	4			4/4	4/4	2/4		Wilm's tumor
	28	18	4					

P: primary; M: metastatic; R: recurrent; V: vimentin; K: keratin; D: desmin; NF: neurofilaments; m: malignant cells; Sa: sarcoma; MM: malignant melanoma; ML: malignant lymphoma; MAb: monoclonal antibodies; Ca-Merkel: neuroendocrine (Merkel cell) skin carcinoma.

* All diagnoses were later confirmed histologically.

† Ca of the stomach (one), SC of the skin (one), Ca (lymphoepithelioma) (one), AdC and AnC primary not known (two).

‡ Ca of the kidney (two), Ca of the thyroid large cell type (two), AnC-sc of the lung (one).

§ All MM with no melanin pigment in the smears.

roblastoma and embryonal rhabdomyosarcoma could be excluded, but a distinction between malignant lymphoma and nonmuscle sarcoma could not be made.

The cytologic diagnosis of germ cell tumors could not be decisively aided by IF typing of tumor cells. The majority of tumor cells in five seminomas were vimentin positive. In three tumors different percentages of K+V− tumor cells were present (*i.e.*, less than 1%, 2%–5%, 10%–20%). Additionally, in one case a few tumor cells coexpressing keratin and vimentin were found (1%). In contrast the majority of tumor cells in three embryonal carcinomas and one teratocarcinoma was keratin positive. In two embryonal carcinomas as well as in the teratocarcinoma there was an additional population of vimentin-positive cells.

Discussion

Our findings document that IF typing of tumor cells in FNA is instrumental in determining the tumor type when combined with the light microscopic findings and clinical data. As detailed above our results are based on more than 400 cases which included the following: (A)

TABLE 5. Cytologic Suggestion of Tumor Type Changed as a Result of Intermediate Filament Typing of Tumor Cells in Fine-Needle Aspirates (Group B)

Case	Age	Site	Initial cytologic diagnosis	IF typing with MAb to			Definitive cytologic diagnosis (LM + IF)	Histologic diagnosis
				V	K	NF		
13	71	LNi	m-ML?	−	+	+	Ca-Merkel	AnC-sc
2	43	Skin	m-ML?	−	+		Ca-Merkel*	AnC-sc
441	26	LNc	m-ML?	−	+		AnC†	AnC-sc
9	24	Thyroid	m-AnC-sc?	+	−		ML	ML
84/A	60	LNc	m-Ca?¶	+	−		ML‡	ML
94/A	44	LNc	m-Ca?¶	+	−		ML	ML
101/A	67	LNa	m-AnC?	+	−		ML‡	ML
102/A	44	Liver	m-Carcinoid?	+	−	−	ML‡§	
221	38	LNc	m-Ca?	+	−		MM‖	MM

IF: intermediate filaments; MAb: monoclonal antibodies; V: vimentin; K: keratin; NF: neurofilaments; LM: light microscopic study; LN: lymph nodes; i: inguinal; c: cervical; a: axillary; m: malignant cells; ML: malignant lymphoma; AnC: anaplastic carcinoma; sc: small cell; Ca: carcinoma; MM: malignant melanoma; LCA: leukocyte common antigen; FNA: fine-needle aspirates.

* Not tested for NF but distinct K positive IF bodies found.

† Tumor cells in FNA also LCA negative.

‡ Tumor cells in FNA also LCA positive.

§ Histologic typing not done; ML diagnosed also in ascites.

‖ Tumor cells with melanosomes documented in transmission electron microscopic study of FNA.

¶ Lymphoepithelioma suggested.

TABLE 6. Ambiguity of Cytologic Diagnosis of Tumor Type Resolved by Intermediate Filament Typing of Tumor Cells in Fine-Needle Aspirates (Group B)

Initial cytologic diagnosis	No. of Cases			IF typing with MAb to				Definitive cytologic diagnosis (LM + IF)*
	P	M	R	V	K	D	NF	
m-Ca? ML?		6		0/6	6/6			Ca†
	22			22/22	0/22			ML
		1		1/1	0/1		0/1	MM‡
m-Ca? Sa?		2		1/2	2/2		1/2	Ca§
	2	3	2	7/7	0/5	1/7		Sa
m-Ca? MM?		2		1/2	2/2			Ca‖
		3		3/3	0/3			MM
m-Ca? Plasmacytoma?	1			1/1	0/1			Plasmacytoma
	1			0/1	1/1			Ca¶
m-ML? Sa?	1			1/1		1/1		Sa
m-ML? Neuroblastoma?	2			2/2			0/2	ML
m-ML? Hepatoblastoma?		1		1/1	0/1			ML
m-Wilms? Sa-rhabdo?	1			1/1	1/1	1/1		Wilm's tumor
	1			1/1	0/1	0/1	0/1	Sa
m-Ewing? Neuroblastoma?	1			1/1		0/1	0/1	Ewing's tumor
m		1			1/1			Ca#
	2			2/2	0/1	1/1	0/1	Sa**
	3			3/3	3/3	2/2	0/1	Wilm's tumor
	1			0/1	0/1	0/1	1/1	Neuroblastoma
	38	19	2					

P: primary; M: metastatic; R: recurrent; V: vimentin; K: keratin; D: desmin; NF: neurofilaments; LM: light microscopic study; MAb: monoclonal antibodies; IF: intermediate filaments; m: malignant cells; Ca: carcinoma; ML: malignant lymphoma; Sa: sarcoma; MM: malignant melanoma; LCA: leukocyte common antigen; FNA: fine-needle aspirates.

* All diagnoses were later confirmed histologically except for two ML where clinical findings and response to therapy were consistent with ML.

† Histologically: small cell anaplastic carcinomas.

‡ Tumor cells in FNA LCA negative; no melanin pigment in the smears.

§ Merkel cell carcinoma (one), Ca: unknown primary (one).

‖ Renal cell carcinoma (one), AdC (one).

¶ Adrenal Ca.

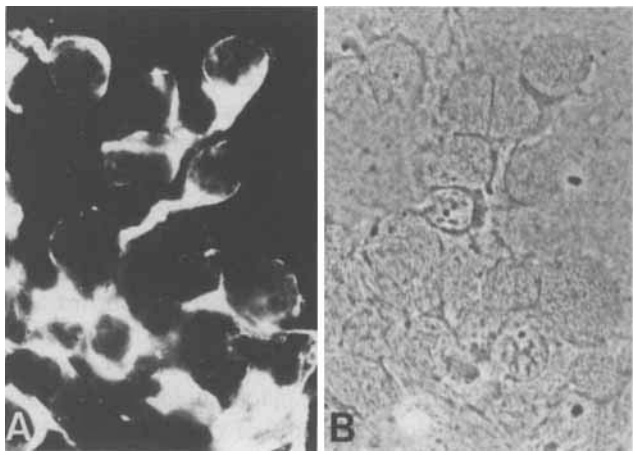
AdC.

** Embryonal rhabdomyosarcoma (one), nonmuscle Sa (one).

271 FNA biopsy specimens collected at random in which the correct and specific cytologic diagnosis of tumor type was apparent from the clinical data and the light microscopic study alone; this group was used to evaluate the reliability of IF tumor typing in various tumor categories as well as to find possible pitfalls in interpretation of results or areas of difficulty; and (B) 132 aspirates which although clearly containing malignant cells had given rise to diagnostic difficulty concerning major tumor categories because of lack of morphologic features indicating differentiation (or cell origin), lack of meaningful clinical data, or both. In these cases differential diagnosis comprised Ca, Sa, MM, ML, seminoma, plasmacytoma, neuroblastoma, Ewing's or Wilm's tumor. Intermediate filament typing of tumor cells in these cases helped to modify the initial cytologic diagnosis.

Limitations of Aspiration Biopsy Cytologic Analysis in Diagnosis of Major Tumor Types

The morphologic diagnosis of tumor type in FNA is in by far the majority of cases accurate and straightforward if malignant cells in the smears show distinct light microscopic features of differentiation, and in addition the clinical setting is appropriate and the diagnosis is made by a well-trained cytopathologist with some years of experience in aspiration cytologic study.[1-4] Quite often even subtypes of particular primary or metastic carcinomas can be revealed.[1-4,8,29]



FIGS. 5A AND 5B. FNA of primary embryonal rhabdomyosarcoma. (A) Tumor cells positively labeled with desmin antibody (indirect immunofluorescence). (B) Phase-contrast picture of cells shown in Figure 5A (×630).

TABLE 7. Cytologic Difficulty in Diagnosis of Tumor Type Not Resolved by Intermediate Filament Typing of Tumor Cells in Fine-Needle Aspirates (Group B)

Record	Age	Site	Cytologic diagnosis	IF typing with MAb to V	K	NF	D	Histologic diagnosis
36/A	27	LNi	m-Sa?	+	−			MM
87/A	47	STT	m-ML? Sa?	+			−	ML
311	1	Retroperitoneum	m	+	−	−	−	Sa-undifferentiated
445	2	Adrenal	m-Neuroblastoma?	−	−	−	−	Neuroblastoma
31/A	1	Adrenal	m-Ca?	−	−	−	−	Ca-adrenal
57/B	28	LNr	m-Seminoma? Ca-embryonal?	+	+			Seminoma
113/B	37	LNc	m-Seminoma? Ca-embryonal?	+	+			Seminoma
266	54	Retroperitoneum	m-Seminoma?	+	−			Seminoma
41	37	Retroperitoneum	m-Seminoma?	+	−			Seminoma
53	34	LNr	m-Seminoma?	+	−			Seminoma
292	19	Retroperitoneum	m-Ca-embryonal?	+	+		−	Ca-embryonal
116	40	Liver	m-Ca-embryonal?	+	+			Ca-embryonal
206	22	LNr	m-Ca-embryonal?	−	+			Ca-embryonal
7/A		LNi	m-Ca?	+	+			Teratocarcinoma

IF: intermediate filament; MAb: monoclonal antibodies; V: vimentin; K: keratin; NF: neurofilaments; D: desmin; LN: lymph nodes; i: inguinal; r: retroperitoneal; c: cervical; STT: soft tissue tumor; m: malignant cells; Sa: sarcoma; Ca: carcinoma; MM: malignant melanoma; ML: malignant lymphoma.

However, the diagnosis of tumor type in FNA (often even if combined with clinical data) clearly has its limitations.[1,6] Particularly in cases in which malignant cells in smears do not display recognizable morphologic features of differentiation the correct diagnosis of the major tumor type can be very difficult. This is best attested by the existence of so-called "difficult aspirates" and is reflected in their diagnoses. Apart from the straightforward diagnosis of malignancy, the cytologic report of these cases usually contains carefully expressed suggestions as to the possible differential diagnosis of tumor type. Various semantic manouvers are used to indicate the different degrees of certainty with which such suggestions are made. The fact is that morphologic diagnosis of tumor type in such cases is based on subjective rather than on objective criteria (since the latter simply do not exist in the smear or are expressed in the way difficult to grasp even by an experienced eye). This is supported by poor interobserver reproducibility among several cytopathologists (irrespective of their experience) in the diagnosis of tumor type in difficult aspirates with no detectable features indicating differentiation of tumor cells[7] and also by poor intraobserver agreement as to malignant cell types.[8]

Our results further confirm this point. In this study the FNA were made by the same cytopathologists who later evaluated morphologic features of cells in the smears. Thus the clinical information obtained by talking to the patient, doctor, radiologist, *etc.*, was taken into account in arriving at the initial cytologic diagnosis. Under these relatively ideal conditions with the group A tumors a small percentage may be misdiagnosed (in our study nine of 271 or 3.3% group A tumors, Table 3). In group B there were still at least 132 aspirates amounting to approximately 2.6% tumors examined over a 2-year period in which the tumor type could not be unequivocally diagnosed (Tables 4–6). On the other hand, as a result of such an approach there were just eight cases (Tables 6 and 7) among 132 difficult aspirates (7%) in which the cytologic diagnosis was limited to malignant cells with no further suggestion of tumor type. In the other 124 cases the one (69 cases) or two (55 cases) suggestions of the most probable tumor type were used to formulate the diagnostic question to be answered by IF typing of tumor cells.

Usefulness of Intermediate Filament Typing of Tumor Cells in Diagnosis of Tumor Type in Histopathologic and Cytopathologic Study

Intermediate filaments have been shown to be differentiation-specific markers, and the diagnostic value of IF typing by means of monoclonal antibodies specific for a single filament type has been recognized and documented in histologic sections of a wide variety of human tissues and tumors by immunofluorescence, immunoperoxidase, or immunoalkaline phosphatase techniques. Carcinomas have been shown to be positive for keratin. Melanomas, malignant lymphomas, and the majority of nonmuscle sarcomas contain only vimentin. Rhabdomyosarcomas and leiomyosarcomas are desmin positive; glial fibrillary acidic protein can be found in tumors of glial origin,

TABLE 8. Modification of Cytologic Diagnosis of Major Tumor Types as a Result of Intermediate Filament Typing of Tumor Cells in Fine-Needle Aspirates (Group B)

Initial diagnosis based on cytologic and clinical data	No. of cases	Final diagnosis based on cytologic and clinical data and IF typing of tumor cells: No. of cases positive for tumor type specified in initial cytologic diagnosis	Final diagnosis based on cytologic and clinical data and IF typing of tumor cells: No. of cases other tumor type diagnosed
1. Positive for Ca	198	189	9 (7 ML, 1 MM, 1 carotid body tumor)
m-suspicious for Ca	18	13	5 (4 ML, 1 MM)
m-suspicious for Ca or other tumor type	45	11	34 (22 ML, 4 MM, 7 Sa, 1 plasmacytoma) (48)
2. Positive for Sa	35	35	0
m-suspicious for Sa	14	14	0
m-suspicious for Sa or other tumor type	12	9	3 (2 Ca, 1 Wilms') (3)
3. Positive for ML	24	24	0
m-suspicious for ML	11	8	3 (3 Ca)
m-suspicious for ML or other tumor type	33	25	8 (6 Ca, 1 MM, 1 Sa) (11)
4. Positive for MM	10	10	0
m-suspicious for MM	9	9	0
m-suspicious for MM or other tumor type	5	3	2 (2 Ca) (2)
5. Positive for malignancy; no tumor type diagnosed or suggested	7		7 (1 Ca, 2 Sa, 3 Wilms', 1 neuroblastoma)

IF: intermediate filament; Ca: carcinoma; m: malignant cells; Sa: sarcoma; ML: malignant lymphoma; MM: malignant melanoma.

whereas tumors of neural origin contain neurofilaments.[10-14,30-37] In general, tumor cells retain the IF characteristic of their cell of origin and do not acquire new IF types in metastases.[10-14,30-37] There are also certain tumor types in which coexpression of two or more IF types is characteristically observed.[38-44] Among so-called small round blue cell malignant tumors of childhood rhabdomyosarcomas can be unequivocally identified with desmin[33,45] and Wilm's tumor by the different cell types expressing keratin, vimentin, and sometimes desmin.[46] The majority of neuroblastomas contain neurofilaments[34-36] whereas Ewing's sarcomas express vimentin.[47]

Several recent studies have suggested that IF typing can also be applied to typing tumor cells in FNA, and that it can improve the diagnosis of tumor type and help to solve common dilemmas of aspiration cytologic study such as differentiation of carcinoma from malignant lymphoma, melanoma, or sarcoma.[5,7,15-18,25,26,48] The diagnostic significance of true coexpression and pseudocoexpression of IF in FNA have been outlined and sources of false coexpression identified.[18] In the current study (1) all tumors finally diagnosed as carcinomas in both groups A and B were keratin positive, (2) all tumors finally diagnosed as sarcomas in both groups were vimentin positive, and (3) all ML and MM in both groups were vimentin positive and those tested for the presence of keratin, also keratin negative.

Influence of Intermediate Filament Typing of Tumor Cells on Diagnosis of Tumor Type in This Series

Table 8 compares the initial diagnosis based on cytologic and clinical data with the final cytologic diagnosis in which results obtained from IF typing of the tumor cells were also included. Of particular interest are those cases where IF typing resulted in a change in the diagnosis of tumor type.

In group A, Table 3 shows that in eight of nine instances in which IF typing of the fine-needle aspirate changed the diagnosis subsequent histologic study supported the change in diagnosis. In the last case (carotid body tumor) the change in diagnosis was supported by arteriography and appearance of the tumor during operation.

In group B—the difficult cases—the most frequent diagnostic dilemma was to distinguish between ML and small cell carcinoma (30% of cases causing diagnostic difficulty) and in vast majority these cases turned out to be ML. Although the numbers of such cases may vary in different laboratories (depending on the type of material which a particular laboratory receives) this very clearly remains the major diagnostic problem of aspiration cytologic study in diagnosing tumor type in small cell anaplastic tumors in adults. Among 18 cases in which cytologic diagnosis or suggestion of tumor type was changed by IF typing of tumor cells there were 11 lymphomas

originally diagnosed or suggested as carcinomas (Tables 3 and 5). Moreover, ML was finally diagnosed in 22 of 29 cases in which the cytologic diagnosis listed both options. It seems that in aspiration cytologic study, as in histopathologic examination,[49] if uncertainty exists between carcinoma or ML the tumor is more likely to be ML.

Our results agree with reports in which vimentin is the only IF type present in ML (although not 100% of the tumor cells are vimentin positive).[26,50,51] They can be contrasted to others in which vimentin was found only in a fraction of the lymphomas that were examined.[12,44,52] The discrepancies in the percentage of lymphomas stained in histologic studies from different laboratories probably lie in the different vimentin antibodies used and/or in the different fixation methods.[53] Malignant lymphoma could be distinguished from Merkel cell carcinoma by IF typing. Tumor cells of the latter tumor coexpress keratin and neurofilaments (Tables 4–6).[25,28] Moreover, IF in Merkel cell carcinoma cells were arranged in distinct IF buttons (for details on differential diagnosis of Merkel cell carcinoma in FNA see Domagala *et al.*[25]).

In 6% of difficult aspirates, carcinoma could not be distinguished from sarcoma by LM. Intermediate filament typing made this distinction possible in all cases since all sarcomas were vimentin positive keratin negative whereas carcinomas were keratin positive. Although the majority of carcinomas do not show coexpression of vimentin some carcinomas do (Table 2, 4, and 6). If true coexpression of keratin and vimentin is found in an otherwise correctly diagnosed metastatic carcinoma of unknown origin, the search for the primary can first be concentrated on those sites where coexpression of keratin and vimentin is characteristic of primary carcinomas.[18,27,39,48] In this series all thyroid, renal, and endometrial carcinomas coexpressed keratin and vimentin in the majority of cells. However, as an increasing number of carcinomas from different sites are studied, coexpression of keratin and vimentin is being reported in a few cases from other sites, although by far the majority of tumors from these sites express only keratin.[30,44] For example in our series coexpression was noted in some cells in three of 13 small cell lung carcinomas, in two of 13 breast carcinomas, and in single examples of adrenocortical, prostatic, and squamous laryngeal carcinoma.

Tumor cells of two sarcoma types, *i.e.*, synovial and epithelioid sarcomas[40–42] (Table 1) as well as the so-called malignant rhabdoid tumor of soft tissue[43] can also coexpress vimentin and keratin. Thus in principle these sarcomas have to be taken into account in differential diagnosis with those carcinomas which coexpress keratin and vimentin. However, (1) these sarcomas are rare, and (2) from practical point of view only carcinomas metastatic to soft tissues would have to be taken into consideration since aspiration biopsy of a metastatic synovial or epithelioid sarcoma without the cytopathologist being aware of the existence or prior removal of the primary tumor would be exceptional.

Clearly, finding coexpression of IF in tumor cells in the smears adds important information relevant to the differential diagnosis of the tumor type but it has also drawbacks especially if light microscopic and clinical information does not permit an unequivocal diagnosis between carcinoma or sarcoma.

Malignant melanoma was readily confirmed or distinguished from carcinoma since tumor cells were only vimentin positive (Tables 1–3).[31] Usually within the framework of clinical and morphologic information MM did not have to be distinguished from ML. This would be impossible by IF typing since both tumors are vimentin positive. However, in rare cases in which this distinction has to be made one may be able to use specific antimelanoma antibodies[54] to confirm MM, and leukocyte common antigen (LCA) to exclude MM and confirm ML.[55,56] In one case (Table 6, top) the unexpected finding of vimentin-positive, keratin-negative, and LCA-negative tumor cells in an aspirate from the lymph node where the differential diagnosis of Ca *versus* ML have been considered, resulted in a diagnosis of MM.

Intermediate filament typing of tumor cells further helped to diagnose some tumors of childhood such as neuroblastoma, Wilm's tumor, Ewing's tumor, and embryonal rhabdomyosarcoma as well as to distinguish between ML and neuroblastoma (Tables 4 and 6). The results of IF typing of tumor cells in some tumors of childhood may pose difficulties in interpretation (see next section).

Limitations of Intermediate Filament Typing in Diagnosis of Tumor Type in Fine-Needle Aspiration

Intermediate filament typing cannot answer all questions in the diagnosis of tumor type in FNA. The cases listed in Table 7 illustrate some situations where difficulties can be expected. They can be divided as follows.

1. As already mentioned, tumors with the same IF content cannot be separated from one another. Thus MM cannot be separated from ML since both tumors are only vimentin positive. The same is true for sarcomas and ML or MM (Table 7) unless the former is a desmin-positive muscle sarcoma. In these instances clearly other markers have to be used, and immunochemistry with markers such as LCA, different lymphocyte-specific antigens, and other surface markers may help to solve these problems.[55–58]

2. One can encounter malignant tumor cells entirely negative for IF.[59] We have two such cases: one adrenocortical carcinoma and one neuroblastoma (Table 7). A cell line isolated from adrenal cortical carcinoma does not express any IF type in the vast majority of the cells.[60] Although the vast majority of neuroblastomas are NF-

positive on frozen sections,[35] NF-negative neuroblastomas have been reported by several groups.[34,36] In cytologic analysis although neuroblastomas which are NF positive are readily diagnosed (Tables 4 and 6) the IF in the scanty cytoplasm of tumor cells can be sparse. Thus in the rare cases of IF-negative neuroblastoma, although a positive diagnosis is not possible, nevertheless such a result excludes the majority of other small round cell tumors of childhood and therefore can be interpreted as indirectly supporting neuroblastoma if the clinical setting is appropriate. One has to be careful not to misdiagnose such a case as ML if there is a significant admixture of vimentin-positive lymphocytes in the aspirate.

3. Differential diagnosis of ML, nonmuscle sarcoma, and Ewing's sarcoma in children is complicated because all cells express vimentin. Whether the recent observation that a few cells in some Ewing's carcinomas express keratin or neurofilaments can be exploited remains to be seen.[61] However, further markers to distinguish these tumors would be clearly very helpful.

4. Currently, IF typing of tumor cells in FNA cannot support the differential diagnosis of germ cell tumors (seminoma, embryonal carcinoma, teratocarcinoma) since conflicting results have been published concerning the presence of keratin and vimentin in tissue sections of tumors from this group.[12,62-65]

5. Technical problems may complicate the interpretation. Aspiration biopsy smears may contain a mixture of tumor cells from different parts of a tumor and in addition may have a variable number of benign cells intermingled among the tumor cells. Intermediate filament typing requires a good quality cellular smear so an appropriate estimate of the percentage of tumor cells positive for IF can be made. The identification of particular cell type, and the correlation of the results in fluorescence with those seen in phase contrast is not a problem for an observer familiar with the light microscopic appearance of cells of a given case but a poor quality smear with only few scattered tumor cells may be misinterpreted. Double immunostaining on the same smear helps to solve some difficulties in interpretation especially in tumors which show coexpression of different IF types. Also, as pointed out earlier, not all monoclonal antibodies against particular IF proteins are equivalent. The importance of a keratin antibody that is really broad specificity should not be underestimated if it is used to diagnose carcinoma; and not all vimentin antibodies yield equivalent staining patterns at least on lymphomas.[53]

Advantages of Intermediate Filament Typing in the Diagnosis of Tumor Type in Fine-Needle Aspiration and Its Significance for the Patient and the Cytopathologist

Intermediate filament typing of tumor cells confirmed the correct cytologic diagnosis of tumor type in 261 cases in group A. One should not underestimate the value of confirming the cytologic diagnosis of tumor type since this confirmation is based on independent objective evidence and therefore it strengthens the certainty of cytologic diagnosis. Routine use of IF typing of tumor cells for confirmation of established cytologic diagnosis in difficult cases can discover a few aspirates where the diagnosis will have to be changed (Table 3), can help to distinguish primary tumor from an unexpected metastasis in a patient with removed and forgotten primary or to distinguish a metastasis from a second primary.[66] Intermediate filament typing of tumor cells can reveal true coexpression of keratin and vimentin in otherwise correctly diagnosed metastatic carcinoma of unknown origin which narrows the search for the primary to those carcinomas known to coexpress these IF. As detailed for the group B tumors in Tables 4 through 6, IF typing is of particular value in cases where a differential diagnosis between major tumor types has to be made, and is in doubt.

Well-performed aspiration biopsy yields at least four but usually more slides. The alcohol fixation routinely used in cytologic study ensures good preservation of IF in tumor cells.

In our routine diagnostic laboratories several smears are left in alcohol until a decision is reached whether IF typing is necessary. If difficulties in tumor typing by light microscopic study are encountered the result of IF typing can be known within 2 hours. This vital information comes when the cytopathologist's interest in solving the diagnostic problem is at its peak. Thus there is an opportunity to learn, since morphologic features of tumor cells can be referred to unequivocally diagnosed tumor types.

This is not to say that cytopathologists cannot learn by comparing morphologic features of tumor cells in difficult cases with the histologic sections from the same cases. But (1) such slides arrive usually weeks (rarely days) after the original cytologic diagnosis had been made; (2) to secure histologic slides of a particular case and compare them with smears, which are already in the files, requires time and energy not everybody can afford for every difficult case; (3) many cytologic laboratories do not have close ties with pathology units; and (4) many patients seek advice in different cities or countries so they disappear from the follow-up. In short, IF typing of tumor cells in aspirates provides the cytopathologist with the opportunity of continuing education based on the most difficult and therefore the most interesting and most informative cases.

Factors Determining the Successful Use of Intermediate Filament Typing of Tumor Cells for the Diagnosis of Tumor Type in Fine-Needle Aspiration

The success of determining the tumor type in aspiration biopsy sample depends on how the IF typing of tumor cells is introduced into the diagnostic process. The more precise the question asked by cytopathologist the better

the chance of unambiguous answer. The question should be based on the analysis of the morphologic features of the tumor cells in smears and the available clinical data. Then IF typing of the tumor cells by a panel of monoclonal antibodies to IF proteins is done. The combined results lead to the final diagnosis. The proper interpretation of results is ensured if the same cytopathologist evaluates morphologic type and the immunocytochemical slides.

Summary

In summary, certainly FNA biopsy is a procedure whose time has come.[3,4,9] However, as pointed out in a recent report which stirred heated debate, "The current enthusiasm for needle aspiration has swept many into an uncritical application of the technique and an unrealistic effort to subclassify tumors without the aid of histology."[6] This study shows that IF typing of tumor cells in needle aspirates adds objective differentiation specific and therefore histogenetically relevant information to the descriptive tumor typing currently used in aspiration cytologic study. It therefore makes efforts to diagnose major tumor types more realistic and certainly more accurate. When combined with morphologic study of tumor cells and clinical information it is an excellent method to refine the cytologic diagnosis of major tumor categories and therefore to prevent error. These findings suggest that diagnostic cytopathologic laboratories should routinely use IF typing of tumor cells in difficult aspirates where the diagnosis of tumor type is in doubt. This would benefit both the patient and the cytopathologist.

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