Immunocytochemical Characterization of Lung Tumors in Fine-Needle Aspiration

The Use of Cytokeratin Monoclonal Antibodies for the Differential Diagnosis of Squamous Cell Carcinoma and Adenocarcinoma

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In the current study, immunocytochemical typing of intermediate filaments was used for a differential diagnosis of human lung tumors from transthoracic fine-needle aspiration biopsies (TFNAB). The authors have compared the cytologic diagnosis of 53 lung cancer cases with the immunofluorescence patterns obtained using a panel of monoclonal antibodies, five of which (KG 8.13, KM 4.62, Ks B.17, KS 8.12, KK 8.60) react with specific cytokeratin polypeptides and one with vimentin (VIM 13.2). Only in six of 23 samples cytologically diagnosed as squamous cell carcinoma did the immunocytochemical typing of cytokeratins (ICTC) confirm the cytologic diagnosis. In seven cases some of the tumor cells stained positively with antibody Ks B.17 specific for simple epithelial keratin (No: 18), suggesting the presence of some cells of glandular origin. In ten additional cases the ICTC was in conflict with the cytologic diagnosis of squamous cell carcinoma (*i.e.*, antibodies Ks 8.12 and KK 8.60 were negative, and antibody Ks B.17, positive) supporting a diagnosis of adenocarcinoma. In 14 of 18 cases cytologically diagnosed as adenocarcinoma, the ICTC confirmed the diagnosis whereas in four cases additional presence of some squamous cells was noticed. The ICTC labeling of cases cytologically diagnosed as undifferentiated and large cell carcinomas was similar to that of the group of adenocarcinomas. Thus, the application of cytokeratin typing for TFNAB samples seems to provide a vital complementation to routine cytologic study, especially for cases cytologically diagnosed as squamous carcinoma.
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IN RECENT YEARS, transthoracic fine-needle aspiration biopsy (TFNAB) has become a most valuable tool for the diagnosis of lung cancer, with minimal risk to the patient.[1–4] However, cytopathology often bears serious limitations with regard to the precise diagnosis due to lack of distinctive histologic features recognizable at the light microscopic level. Thus, in about 20% of poorly differentiated squamous, adenocarcinomas, and large cell carcinomas of the lung, a definitive diagnosis cannot be made by cytopathologic examination alone.[5–8] Nevertheless, cytologic examination, complemented by advanced radiologic techniques, enables the diagnosis of the majority of cases of bronchogenic carcinomas using TFNAB samples into squamous cell carcinomas, adenocarcinomas, large and small cell carcinomas, and undifferentiated carcinomas. This simplified cytopathologic classification is often insufficient with regard to prognosis and choice

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of treatment. Thus, a more precise differential diagnosis of TFNAB specimens of lung tumors is most desirable.

During the last several years, intermediate filament typing has become of great help in differential diagnosis of a large variety of human anaplastic tumors.[9–13] This approach was recently introduced also for fine-needle aspirates, especially in tumors containing a single subfamily of filament subunits.[14–19]

Among the intermediate filaments, the cytokeratin subfamily, which is characteristic for epithelial cells and carcinomas, is the most molecularly diverse.[20–23] About 20 different cytokeratin polypeptides from various human epithelia have been isolated and characterized biochemically, immunochemically as well as genetically.[21,23–26] It has been shown that each type of epithelial cell contains a characteristic combination of cytokeratin polypeptides which may be used to identify the particular cell type either in the normal state or after malignant transformation.[21–23,26–32] A significant progress in the immunohistologic subtyping of carcinomas has recently been made with the development of batteries of antibodies (mostly monoclonal), which enable a differential diagnosis of carcinomas based on their specific cytokeratin expression profile.[22,27–29,33] However, a wide application of such antibodies for differential cytopathologic diagnosis of lung tumors using TFNAB has not yet been made.

In the current study we compare the cytologic diagnosis of various lung tumors with the immunofluorescent labeling obtained using a panel of six monoclonal antibodies, five of them reactive with specific cytokeratin polypeptides and one reactive with vimentin. The results indicated that the immunocytologic labeling is of major importance for a correct diagnosis, especially in tumors cytologically diagnosed as squamous cell carcinomas.

Materials and Methods

In 53 patients with bronchogenic carcinoma the diagnosis was made by TFNAB followed by conventional staining methods combined with immunocytochemical typing of cytokeratins (ICTC). In addition to ICTC, histologic diagnosis of surgically resected tumors from 19 of these patients and ultrastructural examination of ten of the tumor were made. Of the 53 lung cancer patients 38 were men and 15 women with an average age of 65 ± 12 years, and an average smoking history of 47 ± 42 pack/years. Thirty-six patients were of European origin, 13 of Asian–African origin, and four were from Israel (Table 1).

In 23 patients the cytologic diagnosis was squamous cell carcinoma, in 18, adenocarcinoma and in 12 patients, undifferentiated or large cell carcinoma. From each TFNAB two slides were prepared from the first aspirate and fixed immediately in 95% alcohol for 30 minutes and stained by Papanicolaou staining[34] for light microscopic evaluation. Tumor tissue for light microscopic examination was fixed in 4% formaldehyde, embedded in paraffin, sectioned (7 μm) and stained with hematoxylin-eosin. For electron microscopic (EM) study, small blocks of tumor tissue (measuring −2 mm) were fixed in glutaraldehyde, post fixed with 1% osmium oxide (O_sO_4), embedded in Epon, and stained with uranyl acetate and lead citrate according to standard techniques.

For immunocytochemical labeling, the needle from TFNAB was washed with 2 ml of phosphate-buffered saline (PBS), and ten slides were prepared using cytocentrifuge, operated at 10,000 RPM for 5 minutes. Slides were immediately fixed in precooled methanol (−20°C) for 20 minutes followed by 1 minute in precooled acetone (−20°C), and stored at −20°C until used (up to 30 days).

The criteria for cytologic diagnosis of squamous cell carcinoma with keratinization (at the light microscopic level) were based on the findings of large cells with aberrant shapes, intense cytoplasmic eosinophilia or orangeophilia, irregular chromatin, and hyperchromasia of the nucleus. In nonkeratinizing squamous cell carcinomas, the cytoplasm was basophilic or amphophilic. The diagnosis of adenocarcinoma was based on finely vacuolated cytoplasm, clusters of cells lacking cellular or nuclear molding and uniform nucleoli in each cell. Large, round or oval

TABLE 1. Demographic Data of Lung Cancer Patients

Cytologic diagnosis	N	Age (yr)	Male:female	Smoking (pack/yr)	Origin		
					Europe	Asia or Africa	Israel
Squamous cell CA	23	66 ± 12* (36–81)**	18:0	50 ± 47* (0–150)**	15	7	1
Adenoca CA	18	63 ± 11 (48–80)	11:7	26 ± 22 (0–60)	14	2	2
Undifferentiated and large cell CA	12	66 ± 12 (40–79)	9:3	75 ± 39 (20–150)	7	4	1
Total	53	65 ± 12	38:15	47 ± 42	36	13	4

CA: carcinoma; Adenoca: adenocarcinoma.

cells with scant cytoplasm were classified as undifferentiated carcinoma.

The EM criteria for diagnosis of squamous cell carcinoma were the findings of desmosomes and abundance of tonofilaments. Adenocarcinomas displayed microvilli, intercellular canaliculi, lumina, or spaces.[35]

Immunofluorescence Procedure

The mouse monoclonal antibodies (MoAb) used in this study were as follows: (1) antibody KG 8.13, a broad-spectrum cytokeratin antibody which reacts with a relatively broad range of human cytokeratins including polypeptides numbers 1,5,6,7,8,10,11 and 18[36]; (2) antibody KK 8.60, which reacts with cytokeratins 10 and 11; as previously suggested, this antibody might be a specific marker for keratinization[30]; (3) antibody KS 8.12, an antibody which reacts with cytokeratin polypeptides 13 and 16 and stains stratified, nonkeratinizing epithelia as well as squamous carcinomas[30,31]; (4) antibody KM 4.62, which reacts with human cytokeratin no. 19[37] and positively stains both adenocarcinomas and squamous carcinomas; (5) antibody Ks B.17, which reacts with cytokeratin polypeptide no. 18 and stains simple and pseudostratified epithelia, as well as the basal cell layer of several nonkeratinizing stratified epithelia[32]; and (6) VIM 13.2, an antibody which reacts specifically with vimentin and thus stains mesenchymal cells and tissues.

The different monoclonal antibodies (now available from Sigma, St. Louis, MO) were usually applied as undiluted hybridoma culture supernatants. All of these antibodies are incubated with the cells for 45 minutes at room temperature. The secondary antibodies were affinity purified goat antibodies raised against mouse F(ab')2, conjugated to lissamine rhodamine B sulfonyl chloride, as previously described.[38] Aliquots of each antibody were added to the cells and after three washings with PBS, 50 μl of the rhodamine-labeled goat anti-mouse IgG were added for further 30 minutes at room temperature. After three additional PBS washings the cells were mounted in Entellan (Merck, Darmstadt, FRG) and examined with a Zeiss Axiophot X-microscope (Zeiss, Oberkochen, FRG) equipped for epifluorescence observations using X40/1.0 or 100/1.3 oil-immersion Plan Neofluar (Zeiss) objectives.

For immunohistochemical labeling, sections of frozen tissue (4–5 μm thick) were cut using a Frigocut 2700 cryostat (Jung-Reichert, Muenchen, FRG) and processed as previously described.[20,31,32,37,38]

Results

Table 2 summarizes the ICTC and some histologic results in cases cytologically diagnosed as squamous cell carcinomas. These analyses were occasionally complemented by EM examinations as indicated in Table 2.

As shown, six of 23 cases (Cases 1–6) stained positively with MoAb KG 8.13 (Fig. 1B), KM 4.62 (Fig. 1D), Ks 8.12 (Fig. 1E), and KK 8.60 (Fig. 1F), whereas Ks B.17 (Fig. 1C') and VIM 13.2 (not shown) were negative, indicating squamous cell origin, in agreement with the cytologic diagnosis. In one of these cases (Case 3 in Table 2) the cytologic and immunocytochemical diagnoses of squamous cell carcinoma was further confirmed by examination of the tumor tissue by immunohistologic study (Figs. 2A–2F) and EM.

In seven cases (Cases 7–13) the tumor cells stained positively with antibodies KG 8.13, KM 4.62, KS 8.12, and KK 8.60, and in addition some cells were positively labeled with antibody Ks B.17, indicating the presence of tumor cells of glandular origin. In two of these cases (Cases 10 and 12) the cells were positively stained for vimentin with antibody VIM 13.2 as well. It was concluded, therefore, that the diagnosis is suggestive of adenosquamous carcinoma (Fig. 1C). In two of the cases (Cases 9 and 11) the histologic diagnosis of tumor tissue was squamous cell carcinoma, whereas the EM diagnosis was squamous cell carcinoma and adenocarcinoma, respectively. In Case 13 the histologic diagnosis of tumor tissue was bronchioloalveolar carcinoma.

In ten cases (Cases 14–23 in Table 2) tumor cells stained positively with MoAb KG 8.13, KM 4.62, and Ks B.17 whereas Ks 8.12 and KK 8.60 were negative. In three cases (Cases 21–23) vimentin-positive cells were also present. An example of a cytologically diagnosed squamous cell carcinoma, which was positively labeled with antibody Ks B.17 but not with KS 8.12 and KK 8.60 is shown in Figures 3A and 3B. It is apparent that in these cases the ICTC is in conflict with the cytologic diagnosis of squamous cell carcinoma, and is suggestive of adenocarcinoma. In some of these cases the ICTC diagnosis of adenocarcinoma was subsequently confirmed histologically (Cases 18 and 22) and/or by EM (Cases 18 and 20). It is noteworthy that in two other cases (Cases 14 and 20) the histologic diagnosis was undifferentiated large cell carcinoma and squamous cell carcinoma. In the latter case the ICTC diagnosis of adenocarcinoma was supported by EM study. It thus appears that a majority of cases cytologically identified as squamous cell carcinomas, in fact contain glandular tumor cells.

Table 3 summarizes the results obtained with 18 cases of cytologically diagnosed adenocarcinomas. In 14 of these, the tumor cells were positively stained with MoAb KG 8.13 (Fig. 4B), KM 4.62 (Fig. 4D), and Ks B.17, whereas Ks 8.12 (Fig. 4E), KK 8.60 (Fig. 4F), and VIM 13.2 were negative. Two cases (Cases 16 and 17) showed some vimentin-positive cells. Thus, the ICTC largely confirmed the cytologic diagnosis of adenocarcinoma. This was also corroborated by histologic examination of tumor tissues resected from some of these patients. As shown in

TABLE 2. Cytologic, Histologic, and Electron Microscopic Diagnosis With Immunocytochemical Typing of Cytokeratins in Lung Tumor Cells From Transthoracic Fine-Needle Aspiration Biopsy and Tumor Tissue Diagnosed as Squamous Cell Carcinomas (Cytology)

	Monoclonal antibodies tested						Diagnosis				
							TFNAB		Tumor tissue		
Case	VIM13.2	KG8.13[18,19]	KM4.62[19]	Ks.B.17[18]	KS8.12[13,16]	KK8.60[10,11]	CTL	ICTC	ICTC	HIST	EM
1 (35)	–	+	+	–	+	+	SQ	SQ			
2 (39)	–	+	+	–	+	+	SQ	SQ			
3 (103)	–	+	+	–	+	+	SQ	SQ	SQ	SQ	SQ
4 (86)	–	–	+	–	+	+	SQWD	SQ			
5 (72)	–		+	–	+	–	SQWD	SQ			
6 (144)	–	+		–	+	*	SQWD	SQ			
7 (160)	–	+	+	+	+	+	SQ	AD-SQ	AD-SQ	SQMD	
8 (153)	–	+	+	+	+	+	SQ	AD-SQ	AD-SQ	SQWD	
9 (179)	–	+	+	+	+	+	SQWD	AD-SQ	AD-SQ	SQMD	SQ
10 (43)	+†	+	+	+	+	+	SQ	AD-SQ			
11 (111)	–	+	+	*	+	–	SQMD	AD-SQ	AD-SQ	SQMD	AD
12 (53)	+†	+	+	+	*	*	SQPD	AD-SQ			
13 (95)	–	+	+	+	–	*	SQ	AD-SQ	AD-SQ	BR-ALV	
14 (97)	–	+	+	+	–	–	SQPD	AD	AD	UND-LC	
15 (68)	–	+	+	+	–	–	SQ	AD			
16 (83)	–	+	+	+	–	–	SQWD	AD			
17 (56)	–	+	+	+	–	–	SQPD	AD			
18 (102)	–	+	+	+	–	–	SQ	AD	AD	AD	AD
19 (114)	–	+	+	+	–	–	SQ	AD			
20 (112)	–	+	+	*	–	–	SQWD	AD	AD	SQWD	AD
21 (84)	+	+	+	+	–	–	SQPD	AD			
22 (25)	+	+	+	+	–	–	SQPD	AD	AD	AD	
23 (78)	+	–	+	+	–	–	SQWD	AD			

CTL: cytologic; EM: electron microscopic; ICTC: immunocytochemical typing of cytokeratins; TFNB: transthoracic fine-needle aspiration biopsy; SQ: squamous cell CA; AD: adenocarcinoma; AD-SQ: adenosquamous CA; WD: well differentiated; MD: moderately differentiated; PD: poorly differentiated; BR-ALV: bronchiolo–alveolar CA; UND: undifferentiated; HIST: histologic.

* Few cells stained positively.

† Vimentin-positive cells in the samples were found to be cytokeratin KG8.13 negative by double immunolabeling. The origin of these cells is not clear (see text).

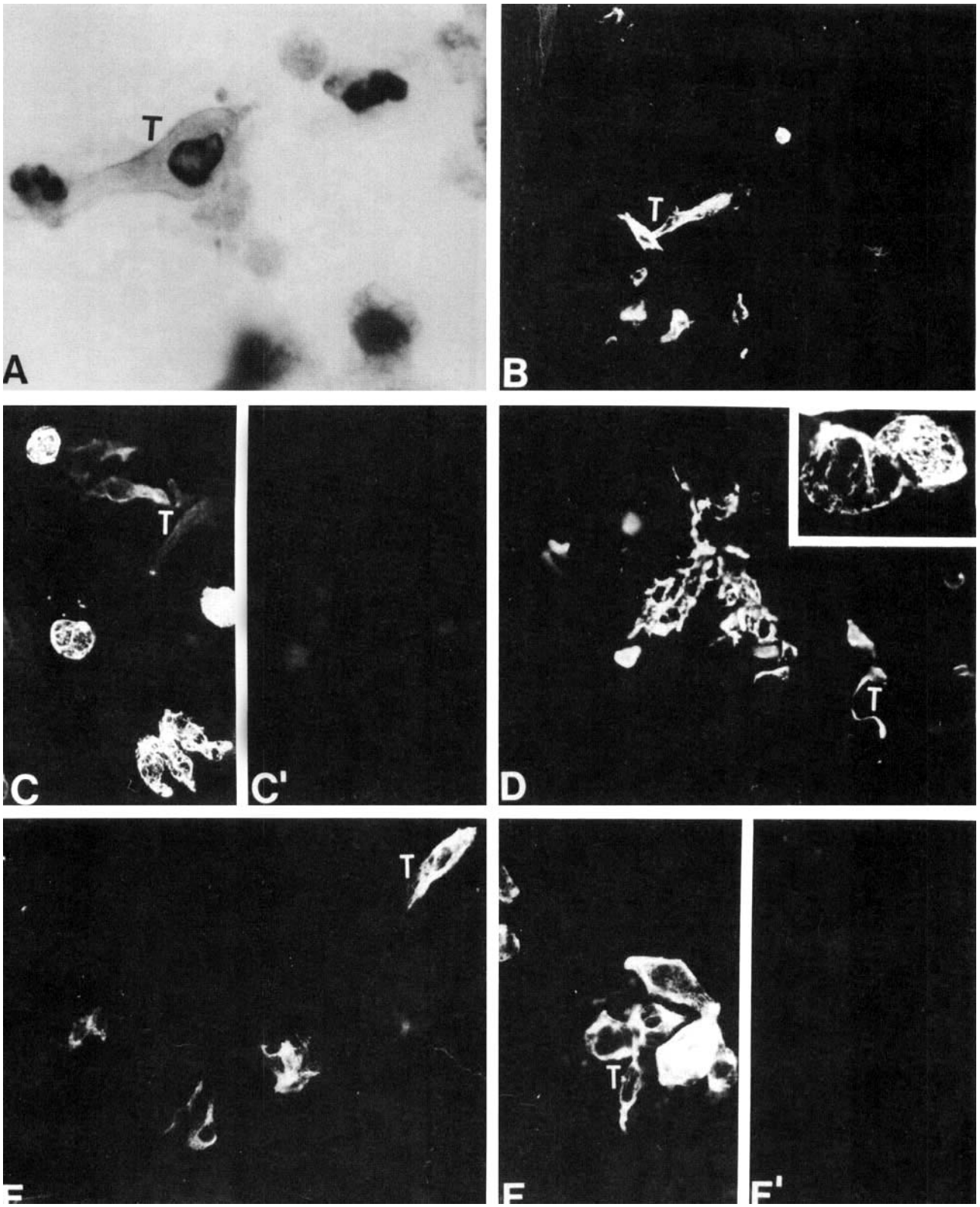
Figures 5A through 5F the histologic appearance and staining patterns were in agreement with a diagnosis of adenocarcinoma. In the same case (Case 2) EM was performed supporting the ICTC and histologic diagnosis of adenocarcinoma. In four cases (Cases 15–18) the ICTC pointed to the presence of some squamous cells suggestive of adenosquamous carcinoma which, most likely, develops due to squamous metaplasia of an originally glandular tumor.

A third category of tumors included 12 cases of cytologically diagnosed undifferentiated and large cell carcinomas (Table 4). Immunocytochemical labelling of these samples was largely similar to that of the group of adenocarcinomas except for one case (Case 1) in which Ks 8.12 and KK 8.60 were both positive, suggesting the presence of squamous cells, possibly due to a metaplastic process. As in the case, ICTC with Ks B.17 was inconclusive, the diagnosis of adenosquamous carcinoma could not be ruled out. In three other cases (Cases 2–4) some cells stained positively with both KS B.17 and KS 8.12 (not shown), therefore, the ICTC may suggest the diagnosis of adenosquamous carcinoma. The histologic and EM diagnoses of Case 2 were of large cell carcinoma and the histologic diagnosis of Case 3 was of mesothelioma. In three cases (Cases 5, 8, and 10) EM confirmed the ICTC diagnosis of adenocarcinoma, whereas the histologic diagnosis of the same tumors was less conclusive (poorly differentiated adenocarcinoma, Case 5), undifferentiated cell carcinoma or thymoma (Case 8), and large cell carcinoma (Case 10).

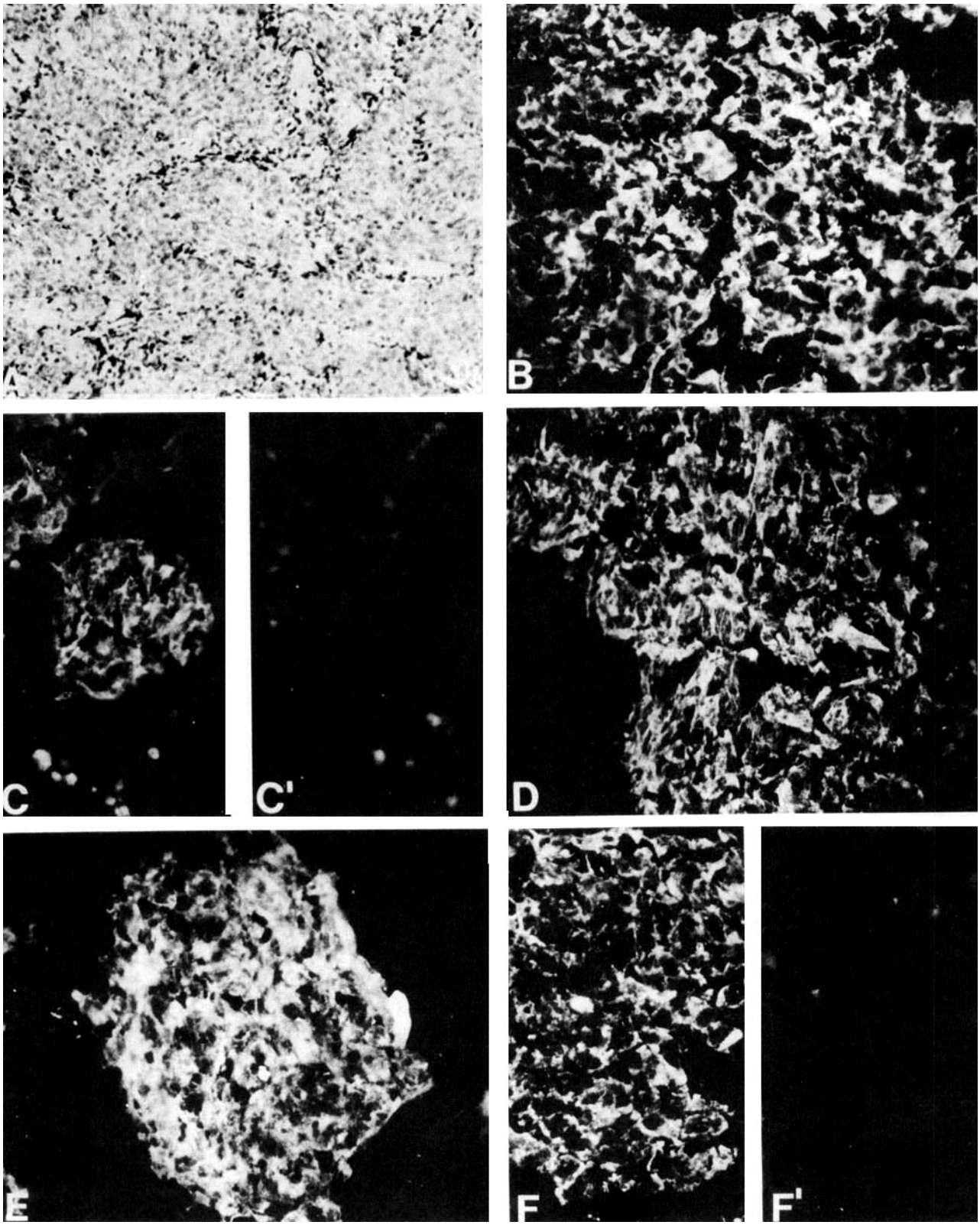
Discussion

The cytologic examination of fine-needle aspiration biopsies of lung tumors has become a widely used diagnostic approach. Its major advantage is the simplicity of the procedure and the minimal risk to the patient.[1–4] Yet, conventional cytologic examination is often handicapped by limited morphologic preservation of distinct cellular structures and the absence of well defined histotypic morphologic features.[39]

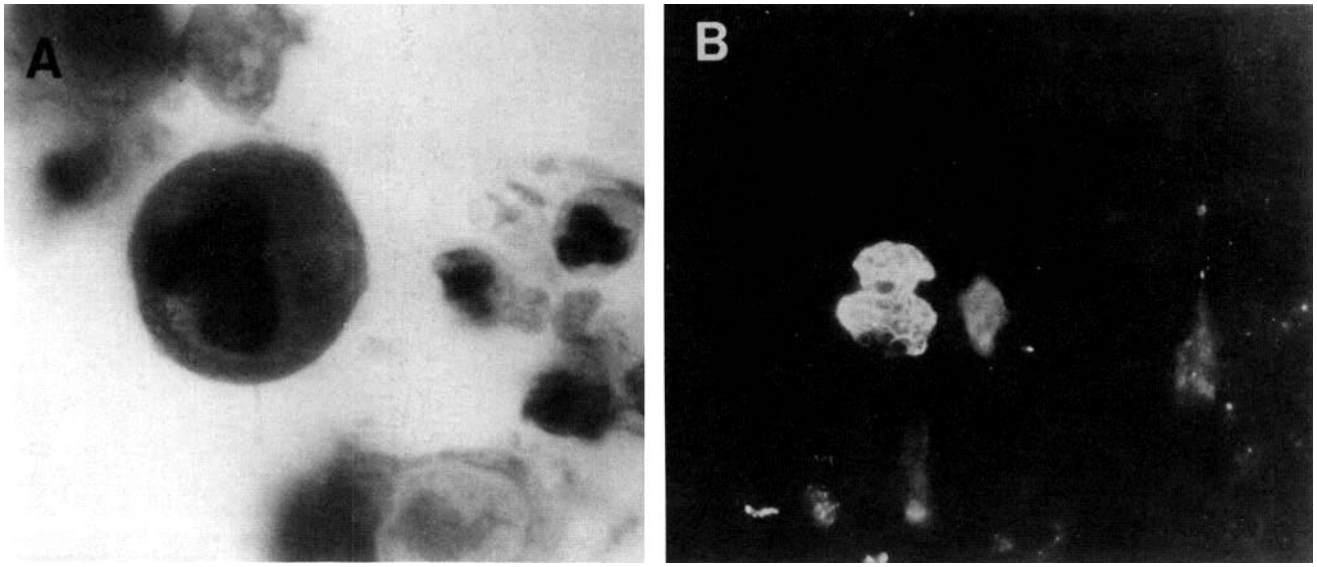
Thus, it had been indicated that in about 20% of poorly differentiated, squamous, adenocarcinomas and large cell carcinomas of the lung, a definitive diagnosis cannot be reached by cytopathologic study alone.[5–8] Moreover, Johnston and Frable[6] showed that in 30% of cytologically diagnosed squamous cell carcinomas (from TFNAB), the diagnosis was not confirmed by subsequent tissue biopsies.



FIGS. 1A–1F. (A) Papanicolaou staining (×1000) and (B, C, C', D, E, F, F') immunocytochemical characterization (×400) of TFNAB of Cases 3 and 11, Table 2, both of which were cytologically diagnosed as squamous cell carcinoma of the lung. The MoAbs used were KG 8.13 (B), Ks B.17 (C, C'), KM 4.62 (D), KS 8.12 (E), and KK 8.60 (F, F'). Antibodies KG 8.13, KM 4.62, and Ks 8.12 uniformly stained the different cell types of both tumors whereas antibody Ks B.17 stained only cells in the moderately differentiated squamous cell carcinoma, Case 11 (C). The latter MoAb was negative on the tadpole (T) cells of the well-differentiated squamous cell carcinoma, Case 3 (C'). In contrast, antibody KK 8.60 stained the well-differentiated squamous cell carcinoma (F) and did not stain the moderately differentiated squamous cell carcinoma (F'). Notice the fibrillar staining in the insert, D, shown in larger magnification (×1000).



FIGS. 2A–2F. (A) Hematoxylin and eosin staining (×100) and (B, C, C', D, E, F, F') (×400) immunohistochemical characterization of tissue blocks from Cases 3 and 11, Table 2, both of which were cytologically diagnosed as squamous cell carcinomas of the lung. The MoAb used were KG 8.13 (B), Ks B.17 (C, C'), KM 4.62, KS 8.12 (E), and KK 8.60 (F, F'). Notice that antibodies KG 8.13, KM 4.62, and KS 8.12 uniformly stained both squamous cell carcinomas. Antibody Ks B.17 stained only the moderately differentiated squamous cell carcinoma, Case 11 (C) and not the well-differentiated squamous cell carcinoma, Case 3 (C'). In contrast, antibody KK 8.60 was positive on the well-differentiated squamous cell carcinoma, Case 3 (F) and negative on the moderately differentiated squamous cell carcinoma, Case 3 (F') (×400).



FIGS. 3A AND 3B. (A) Papanicolaou staining (×1000) and (B) immunofluorescent labeling with antibody Ks B.17 (×400) of TFNAB (Table 2, Case 112). No staining of the cells was obtained with antibodies KS 8.12 or KK 8.60 (not shown).

Naturally, a requirement for supporting histologic examination renders the routine use of TFNAB less attractive. It is thus apparent that the reliability of cytologic examinations should be critically evaluated and corroborated, whenever possible, by complementary tests which may be performed on TFNAB samples and provide more specific and definitive cell typing.

The approach applied here was based on the immunocytologic labeling of TFNAB samples with a variety of monoclonal antibodies reacting with intermediate filaments (especially with different cytokeratin polypeptides) for the differential diagnosis of adenocarcinomas and squamous cell carcinomas. Such diagnosis is of a major practical importance since these two classes of tumors have markedly different prognoses and recommended treatments.[40]

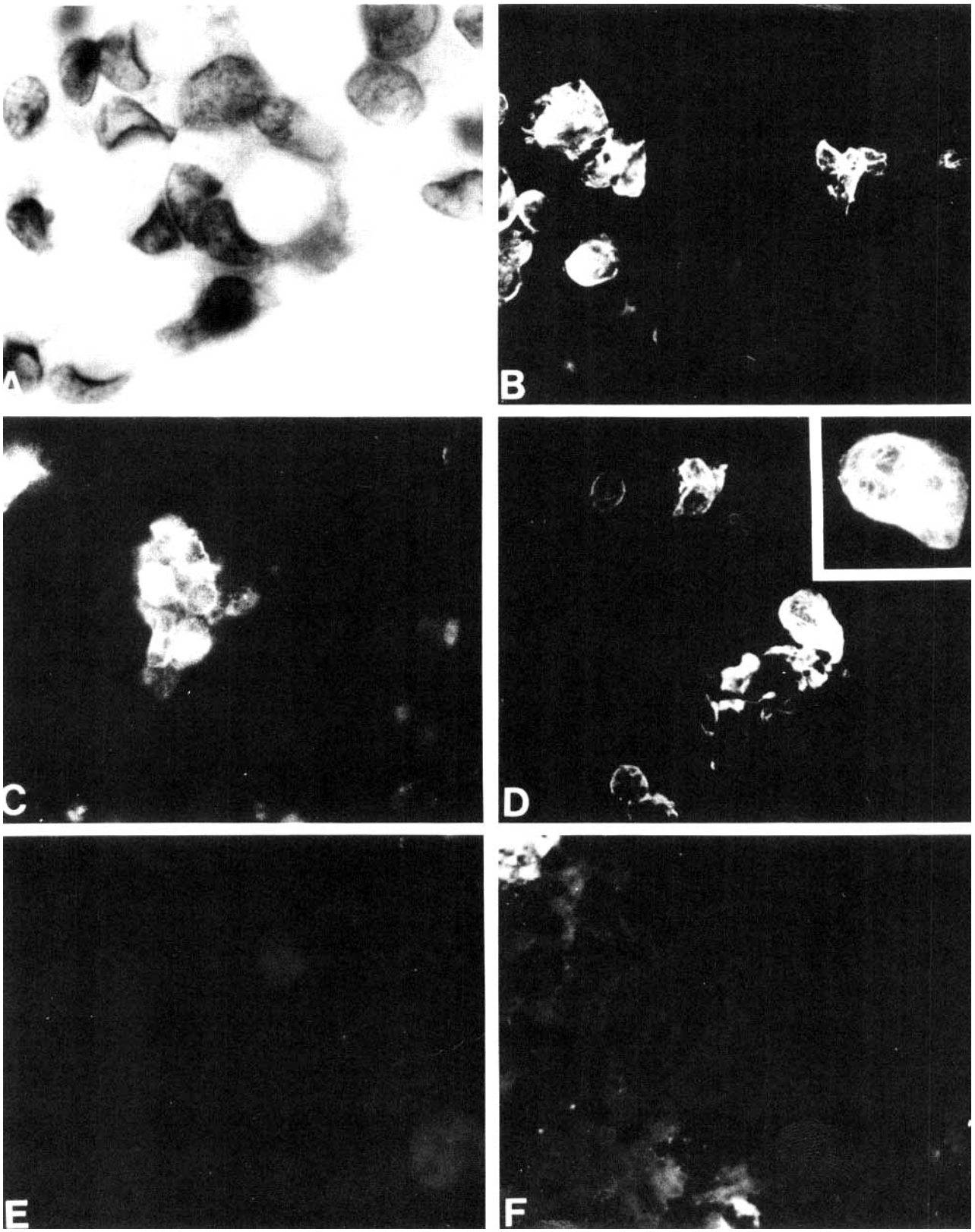
TABLE 3. Cytologic, Histologic, and Electron Microscopic Diagnosis With Immunocytochemical Typing of Cytokeratins in Lung Tumor Cells From Transthoracic Fine-Needle Aspiration Biopsy and Tumor Tissue Diagnosed as Adenocarcinomas (Cytology)

							Diagnosis				
	Monoclonal antibodies tested						TFNAB		Tumor tissue		
Case	VIM13.2	KG8.13[18,19]	KM4.62[19]	Ks.B.17[18]	KS8.12[13,16]	KK8.60[10,11]	CTL	ICTC	ICTC	HIST	EM
1 (22)	–	+	+	+	–	–	AD	AD			
2 (106)	–	+	+	+	–	–	AD	AD	AD	AD	AD
3 (32)	–	+	+	+	–	–	AD	AD			
4 (21)	–	+	+	+	–	–	AD	AD			
5 (38)	–	–	+	+	–	–	AD	AD			
6 (61)	–*		+	+	–	–	AD	AD			
7 (24)	–	+	–	+	–	–	AD	AD			
8 (138)	–	+	+	+	–	–	AD	AD			
9 (130)	–	+	+	+	–	–	AD	AD			
10 (117)	–	+		+	–	–	AD	AD			
11 (123)	–	+		+	–	–	AD	AD			
12 (158)	–	+	+	+	–	–	AD	AD	AD	UND	
13 (131)	–	+	†	†	–	–	ADPD	AD/UND			
14 (122)	–	+	+	+	–	–	AD	AD			
15 (118)	–	+		+	–	+	AD	AD-SQ			
16 (116)	+	+	+	+	–	+	AD	AD-SQ			
17 (73)	+	–	+	+	†	–	AD	AD-SQ			
18 (178)	–	+	+	+	+	+	AD	AD-SQ	AD-SQ	AD	

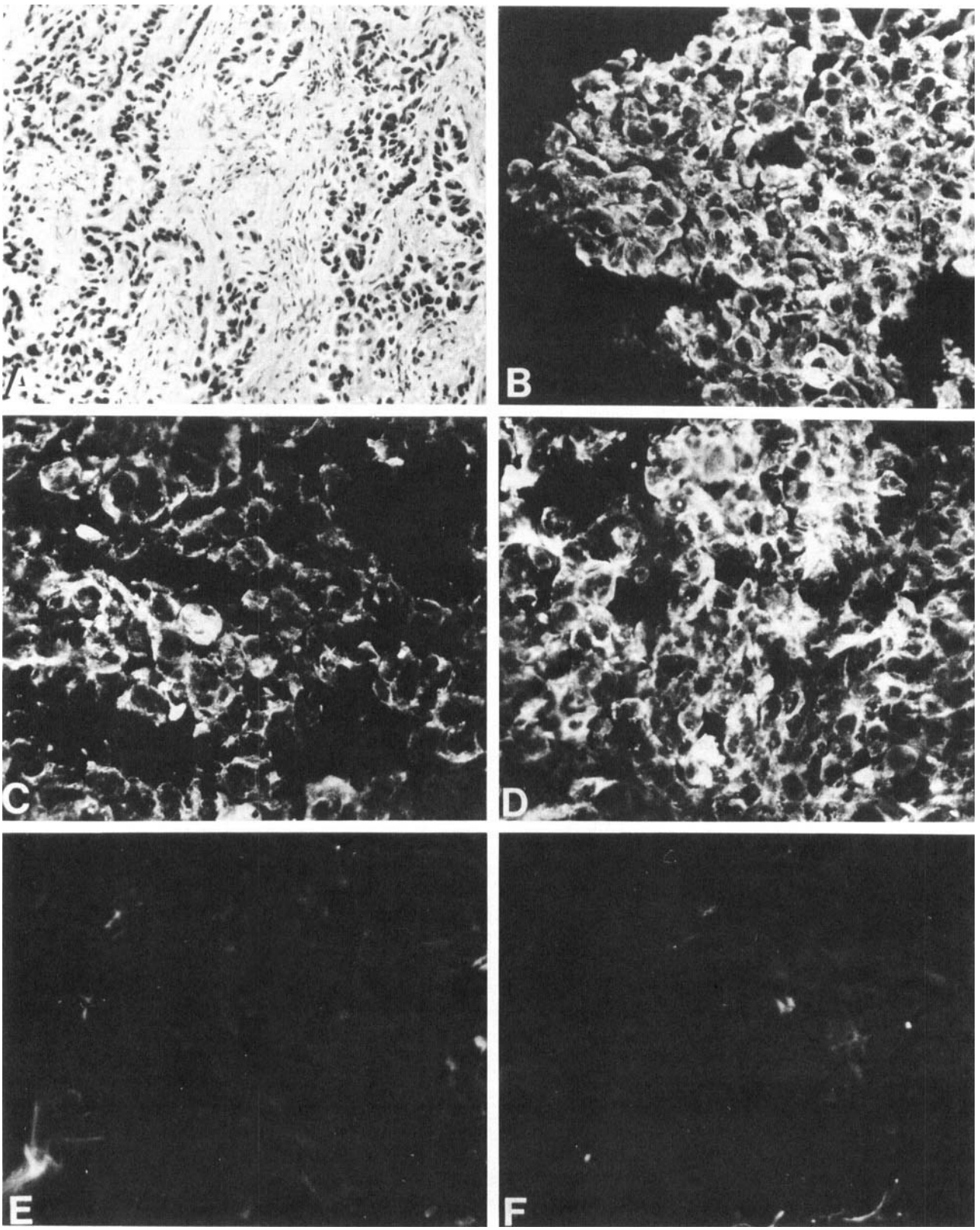
CTL: cytologic; EM: electron microscopic; ICTC: immunocytochemical typing of cytokeratins; TFNAB: transthoracic fine-needle aspiration biopsy; AD: adenocarcinoma; CA: carcinoma; AD-SQ: adenosquamous cell CA; UND: undifferentiated CA; HIST: histologic.

* Vimentin-positive cells in the samples were found to be cytokeratin KG8.13 negative by double immunolabeling. The origin of these cells is not clear (see text).

† Few cells stained positively.



FIGS. 4A–4F. (A) Papanicolaou staining (×1000) and (B, C, D, E, F) immunocytochemical characterization (×400) of TFNAB of Case 2, Table 3, which was cytologically diagnosed as adenocarcinoma of the lung. The MoAb used were KG 8.13 (B), Ks B.17 (C), KM 4.62 (D), KS 8.12 (E), and KK 8.60 (F). Notice that antibodies KG 8.13, KM 4.62, and Ks B.17 uniformly stained the tumor cells, whereas antibodies Ks 8.12 and KK 8.60 were negative on these cells. Notice also the fibrillar staining in the larger magnification (insert in D, ×1000).



FIGS. 5A–5F. (A) Hematoxylin and eosin staining (×100) and immunohistochemical characterization of tissue blocks from Case 2, Table 3, which was cytologically diagnosed as adenocarcinoma of the lung. The MoAb used were KG 8.13 (B), Ks B.17 (C), and KM 4.62 (D). Ks B.17 uniformly stained the tumor, whereas antibodies KS 8.12 and KK 8.60 were negative (B–F, ×400).

TABLE 4. Cytologic, Histologic, and Electron Microscopic Diagnosis With Immunocytochemical Typing of Cytokeratins in Lung Tumor Cells From Transthoracic Fine-Needle Aspiration Biopsy and Tumor Tissue Diagnosed as Undifferentiated Carcinomas

	Monoclonal antibodies tested						Diagnosis				
							TFNAB		Tumor tissue		
Case	VIM13.2	KG8.13[18,19]	KM4.62[19]	KS.B.17[18]	KS8.12[13,16]	KK8.60[10,11]	CTL	ICTC	ICTC	HIST	EM
1 (19)	–	+	+		+	+	UND	SQ/AD-SQ			
2 (181)	–	+	+	+	+	–	LC	AD-SQ	AD-SQ	LC	LC
3 (173)	–	+	+	+	+	+	UND	AD-SQ	AD-SQ	ME	
4 (67)	–	+	+	*	*	–	UND	AD-SQ			
5 (100)	–	+	+	+	–	–	UND	AD	AD	ADPD	AD
6 (51)	–	+	+	+	–	–	UND	AD			
7 (94)	–		+	+	–	–	UND	AD			
8 (81)	+†	+	+	+	–	–	UND/TH	AD	AD	UND/TH	ADPD
9 (69)	–		+	–	–	–	LC	UND			
10 (108)	–	+	+	+	–	–	LC	AD	AD	LC	AD
11 (139)	–	+	+	+	–	–	UND	AD	*AD*	*UND*	
12 (132)	–	+	+	+	–	–	UND	AD			

CTL: cytologic; EM: electron microscopic; ICTC: immunocytochemical typing of cytokeratins; TFNAB: transthoracic fine-needle aspiration biopsy; AD: adenocarcinoma; CA: carcinoma; AD-SQ: adeno-squamous cell CA; SQ: squamous cell CA; UND: undifferentiated CA; LC: large cell CA; PD: poorly differentiated; TH: thymoma; ME: mesotelioma; HIST: histologic.

* Few cells stained positively.

† Vimentin-positive cells in the samples were found to be cytokeratin KG8.13 negative by double immunolabeling. The origin of these cells is not clear (see text).

Comparison of the cytologic results to those obtained by cytokeratin labeling indicated that while the diagnosis of adenocarcinomas by the former approach was largely confirmed by immunolabeling, the majority of tumors cytologically identified as squamous carcinomas turned out to be adenocarcinomas or mixed adenosquamous carcinomas. In some of these cases the validity of the immunocytochemical results was further confirmed by histologic examination of the surgically resected primary lung tumor or by EM analysis. It was therefore concluded that a routine labeling of TFNAB samples with antibodies such as KS 8.12, KK 8.60, and Ks B.17 (which can distinguish adenocarcinomas from squamous carcinomas) is of a great potential in diagnostic value.

Another related conceptual problem which might be considered here concerns the cellular origin of squamous cells in lung carcinomas. Since normal lungs do not contain any *bona fide* squamous epithelium, presence of squamous elements in lung tumors is indicative of a process whereby simple or pseudo stratified epithelia undergo metaplasia and adopt a squamous differentiation. The temporal (and causal) interrelationships between the appearance of such squamous metaplasia and a neoplastic transformation are not clear. Yet, one may speculate that "pure" squamous carcinomas (*i.e.*, tumors in which no simple epithelial cells are detected by labeling with MoAb Ks B.17) originate in a squamous metaplastic lesion in the lungs. The possibility that such lesions are indeed preneoplastic in nature is widely accepted.[41] In contrast, those cases in which both simple and stratified epithelial cells are detected in the TFNAB sample may originate in the nonsquamous respiratory epithelium which later undergo squamous metaplasia. It remains now to be determined whether these mixed tumors and the pure adenocarcinoma differ in their clinical manifestations and prognosis.

The origin of the few vimentin-positive cells occasionally detected in TFNAB of cases cytologically diagnosed as either squamous, adenocarcinomas, or poorly differentiated carcinomas is not clear. Two possibilities may, however, be considered: (1) double expression (vimentin/cytokeratin) in some of the tumor cells (see Domagala *et al.*[19]); or (2) presence of nonmalignant cells of mesenchymal or leukocytic origin. Double labeling experiments carried out on three cases (Cases 10, 12, 22, Table 2) indicated that the vimentin-containing cells were not labeled for cytokeratin (not shown) and cytologic examination excluded the possibility that these cells were leukocytes. Therefore, it seems most likely that these cells are stromal fibroblasts present within the tumor. It is noteworthy that Upton *et al.*[42] indicated that adenocarcinomas but not squamous cell carcinomas frequently express vimentin in addition to cytokeratin in agreement with our results.

In conclusion, the application of cytokeratin typing for TFNAB samples seems to provide a vital complementation to the routine cytologic study. Thus in about 70% of the cases originally diagnosed as squamous cell carcinomas (ranging from poorly to well differentiated) the cytologic diagnosis was apparently incorrect. The immunocytochemical labeling in those cases is, therefore, of primary importance for the correct prognosis and selection of an optimal therapy.[40]

REFERENCES

1. Thornbury JR, Burke DP, Naylor B. Transthoracic needle aspiration biopsy: Accuracy of cytologic typing of malignant neoplasm. *Am J Radiol* 1981; 136:719–724.

2. Lalli AF, McCormack LJ, Zelch M, Reich NE, Belovich D. Aspiration biopsies of chest lesions. *Radiology* 1978; 127:35–40.

3. Sagel SS, Ferguson TB, Forrest JV, Roper CL, Weldon CS, Clark RE. Percutaneous transthoracic aspiration needle biopsy. *Ann Thorac Surg* 1978; 26:399–405.

4. Sargent EN, Turner AF, Gordonson J, Schwinn CP, Pashky O. Percutaneous pulmonary needle biopsy: Report of 350 patients. *Am J Radiol* 1978; 122:758–768.

5. Hess FG Jr, McDowell EM, Trump BFP. Pulmonary cytology: Current status of cytologic typing of respiratory tract tumors. *Am J Pathol* 1981; 103:323–333.

6. Johnston WW, Frable WJ. Cytopathology of the respiratory tract: A review. *Am J Pathol* 1976; 84:372–424.

7. Koss LG. Cancer of the lung. In: Diagnostic Cytology and its Histopathologic Bases, ed. 2. Philadelphia: JB Lippincott, 1968; 338–385.

8. Frost JK. Cancers without identifiable functional differentiation: The cell in health and disease. An evaluation of cellular morphologic expression of biologic behavior. In: Wied GL, ed. Clinical Cytology, vol. 2. Baltimore: Williams and Wilkins, 1969; 130–136.

9. Ramaekers FCS, Puts JJG, Kant A, Moeskser O, Jap PHK, Vooijs GP. Use of antibodies to intermediate filaments in the characterization of human tumors. *Cold Spring Harbor Symp Quant Biol* 1981; 46:331–339.

10. Osborn M, Weber K. Tumor diagnosis by intermediate filament typing: A novel tool for surgical pathology. *Lab Invest* 1983; 48:372–394.

11. Gabbiani G, Kapanci Y, Barazzone P, Franke WW. Immunochemical identification of intermediate-sized filaments in human neoplastic cells: A diagnostic aid for the surgical pathologist. *Am J Pathol* 1981; 104:206–216.

12. Osborn M, Altmannsberger M, Debus E, Weber K. Conventional and monoclonal antibodies to intermediate filament proteins in human tumor diagnosis: I. The transformed phenotype. In: Levine A, Topp W, Vande Woude G, Watson JD, eds. Cancer Cells. New York: Cold Spring Harbor Laboratories, 1984; 191–200.

13. Vogel AM, Gown AM. Monoclonal antibodies to intermediate filament proteins: Use in diagnostic surgical pathology. In: Shay J, ed. Cell and Muscle Motility. New York: Plenum, 1984; 397–402.

14. Altmannsberger M, Osborn M, Droese M, Weber K, Schauer A. Diagnostic value of intermediate filament antibodies in clinical cytology. *Klin Wochenschr* 1984; 62:114–123.

15. Ramaekers F, Haag D, Jap P, Vooijs PG. Immunochemical demonstration of keratin and vimentin in cytologic aspirates. *Acta Cytol* 1984; 28:385–392.

16. Domagala W, Lubinsky J, Weber K, Osborn M. Intermediate filament typing of tumor cells in fine needle aspirates by means of monoclonal antibodies. *Acta Cytol* 1986; 30:214–224.

17. Domagala W, Weber K, Osborn M. Differential diagnosis of lymph node aspirates by intermediate filament typing of tumor cells. *Acta Cytol* 1986; 30:225–234.

18. Domagala W, Lubinski J, Lasota J *et al.* Decisive role of intermediate filament typing of tumor cells in the differential diagnosis of difficult fine needle aspirates. *Acta Cytol* 1987; 31:253–266.

19. Domagala W, Weber K, Osborn M. Diagnostic significance of coexpression of intermediate filaments in fine needle aspirates of human tumors. *Acta Cytol* 1988; 32:49–59.

20. Franke WW, Appelhans B, Schmid E, Freudenstein C, Osborn M, Weber K. Identification of characterization of epithelial cells in mammalian tissues by immunofluorescence microscopy using antibodies to prekeratin. *Differentiation* 1979; 15:7–25.

21. Moll R, Franke WW, Schiller DL, Geiger B, Krepler R. The catalog of human cytokeratins: Patterns of expression in normal epithelia, tumors, and cultured cells. *Cell* 1982; 31:11–24.

22. Moll R, Krepler R, Franke WW. Complex cytokeratin polypeptide patterns observed in certain human carcinomas. *Differentiation* 1983; 23:256–269.

23. Quinlan RA, Schiller DL, Hatzfeld M *et al.* Patterns of expression and organization of cytokeratin intermediate filaments. *Ann NY Acad Sci* 1986; 455:282–306.

24. Fuchs E, Hanukoglu I, Marchuk D, Grace P, Kim HK. The nature and significance of differential keratin gene expression. *Ann NY Acad Sci* 1986; 455:436–450.

25. Schiller DL, Franke WW, Geiger B. A subfamily of relatively large and basic cytokeratin polypeptides as defined by peptide mapping is represented by one or several polypeptides in epithelial cells. *EMBO J* 1982; 1:761–769.

26. Cooper D, Shermer A, Pruss R, Sun TT. The use of alF, AE1 and AE3 monoclonal antibodies for the identification and classification of mammalian epithelial keratins. *Differentiation* 1984; 28:30–35.

27. Moll R, Franke WW. Cytochemical cell typing of metastatic tumors according to their cytoskeletal proteins. In: Lapis K, Liotta LA, Robson AS, eds. Biochemistry and Molecular Genetics of Cancer Metastasis. Gravenhag, The Netherlands: Marinus Nijhoff, 1986; 101–113.

28. Moll R. Diversity of cytokeratin in carcinomas. *Acta Histochem [Suppl] (Jena)* 1987; 34:37–44.

29. Blobel GA, Moll R, Franke WW, Vogt-Moykopf I. Cytokeratins in normal lung and lung carcinomas: I. Adenocarcinomas, squamous cell carcinomas, and cultured cell lines. *Virchows Arch [Cell Pathol]* 1984; 45:407–429.

30. Huszar M, Gigi-Leitner O, Moll R, Franke WW, Geiger B. Polypeptide-specific monoclonal cytokeratin antibodies in the differential diagnosis of squamous carcinomas and adenocarcinomas. *Differentiation* 1986; 31:141–153.

31. Geiger S, Geiger B, Leitner O, Marshak G. Cytokeratin polypeptides expression in different epithelial elements of human salivary glands. *Virchows Arch [A]* 1987; 410:403–414.

32. Marshak G, Leitner O, Geiger B. Cytokeratin polypeptide expression during the histogenesis of guinea pig submandibular salivary gland development. *Development* 1987; 100:699–711.

33. Debus E, Moll R, Franke WW, Weber K, Osborn M. Immunohistochemical distinction of human carcinomas by cytokeratin typing with monoclonal antibodies. *Am J Pathol* 1984; 114:121–130.

34. Papanicolaou GN. A new procedure for staining vaginal smears. *Science* 1942; 95:438–439.

35. Ghadially FN. Is it an adenocarcinoma or a squamous cell carcinoma. In: Ghadially FN, ed. Diagnostic Electron Microscopy of Tumours, ed. 2. London: Butterworths, 1985; 87–95.

36. Gigi O, Geiger B, Eshhar Z *et al.* Detection of a cytokeratin determinant common to diverse epithelial cells by a broadly cross reacting monoclonal antibody. *EMBO J* 1982; 1:1429–1437.

37. Gigi-Leitner O, Geiger B. Antigenic interrelationship between 40 KD cytokeratin polypeptide and desmoplakins. *Cell Motil Cytoskel* 1986; 6:628–639.

38. Brandtzaeg P. Conjugates of immunoglobulin G with different fluorophores: I. Characterization by anionic exchange chromatography. *Scand J Immunol* 1973; 2:273–290.

39. Pilotti S, Rilke F, Gribaudi G, Damascelli B. Fine needle aspiration biopsy cytology of primary and metastatic pulmonary tumors. *Acta Cytol* 1982; 26:661–666.

40. Bruderman I, Bronchogenic carcinoma. In: Baum GL, Wolinsky E, eds. Textbook of Pulmonary Diseases, ed. 4. Boston: Little, Brown & Co., 1989; 1201–1210.

41. Spencer H. Carcinoma of the Lung. In: Pathology of the Lung. Oxford: Pergamon Press, 1977; 793–749.

42. Upton PM, Hirohashi S, Tome Y, Miyazawa N, Suemasu K, Shimosato Y. Expression of vimentin in surgically resected adenocarcinomas and large cell carcinomas. *Am J Surg Pathol* 1986; 10:560–567.