Typical and Atypical Pulmonary Carcinoid Tumor Overdiagnosed as Small-Cell Carcinoma on Biopsy Specimens

A Major Pitfall in the Management of Lung Cancer Patients

Giuseppe Pelosi, MD, Jaime Rodriguez, MD,† Giuseppe Viale, MD,* and Juan Rosai, MD†*

Abstract: Seven patients with typical or atypical pulmonary carcinoid tumors overdiagnosed as small-cell carcinoma on bronchoscopic biopsies are described. Bronchial biopsies from 9 consecutive small-cell lung carcinoma patients were used as control group for histologic and immunohistochemical studies (cytokeratins, chromogranin A, synaptophysin, Ki-67 [MIB-1], and TTF-1). The carcinoid tumors presented as either central or peripheral lesions composed of tumor cells with granular, sometimes coarse chromatin pattern, high levels of chromogranin A/synaptophysin immunoreactivity, and low (<20%) Ki-67 (MIB-1) labeling index. The tumor stroma contained thin-walled blood vessels. Small-cell carcinomas always showed central tumor location, finely dispersed nuclear chromatin, lower levels of chromogranin A/synaptophysin, and high (>50%) Ki-67 (MIB-1) labeling index. The stroma contained thick-walled blood vessels with glomeruloid configuration. Judging from this study, overdiagnosis of carcinoid tumor as small-cell carcinoma in small crushed bronchial biopsies remains a significant potential problem in a worldwide sample of hospital settings. Careful evaluation of hematoxylin and eosin sections remains the most important tool for the differential diagnosis, with evaluation of tumor cell proliferation by Ki-67 (MIB-1) labeling index emerging from our review as the most useful ancillary technique for the distinction.

Key Words: carcinoid, small-cell carcinoma, lung, Ki-67

(*Am J Surg Pathol* 2005;29:179–187)

Pulmonary carcinoid tumors, either typical or atypical, are rare neuroendocrine neoplasm, which account for 1% to 2% of all lung malignancies, whereas small-cell lung carcinoma is one of the most prevalent types of lung carcinoma, together with adenocarcinoma and squamous cell carcinoma.[14,68] Pulmonary carcinoid tumors were first identified as such by Hamperl in 1937,[28] having previously included among the "bronchial adenomas" together with several types of salivary gland-related low-grade tumors characterized by an indolent clinical course.[8,47,50,74] Pulmonary carcinoid tumors are currently included in a three-tier clinicopathologic spectrum of neoplasms of the lung with neuroendocrine differentiation, ranging from low-grade tumors with a long life expectancy (typical carcinoid tumors) to intermediate-differentiation tumors with a more aggressive clinical course (atypical carcinoid tumors), to high-grade neoplasms with a dismal prognosis (small-cell and large-cell neuroendocrine carcinomas).[5,68–70]

Since small biopsy fragments obtained at the time of fiberoptic bronchoscopy are commonly used for the diagnosis of lung cancer patients, a reliable and reproducible segregation of carcinoid tumors from small-cell carcinomas is mandatory to appropriately manage these patients. Complete surgical resection is the therapy for choice for carcinoid tumors,[49] whereas small-cell carcinomas are nearly always treated by aggressive combination chemotherapy alone,[56,58,61] except for the rare examples presenting as a peripheral lung nodule,[13,14] for which surgical eradication has been proposed.[60]

Over the years, several reports have commented on the risk of overinterpreting lower respiratory tract carcinoid tumors, either typical[31,43,45,52,60,75] or atypical,[20,25,39,60,66,76] as small-cell carcinomas in small biopsy tissue fragments[18,25,31,39,43,45,60,66,75,76] or cytologic samples.[20,39,43,48,52,65] Very few studies, however, have focused on this differential diagnosis and discussed in detail the criteria to be used on biopsy specimens to avoid this pitfall. Only recently, Aslan et al have reported in an abstract form that the assessment of the tumor proliferative fraction may aid in distinguishing pulmonary carcinoid tumors from small-cell carcinomas in bronchial biopsies having extensive crush artifacts.[4]

The aim of the current paper is to review a series of pulmonary carcinoids, either typical or atypical, that were preoperatively overdiagnosed as small-cell carcinomas, and to evaluate the usefulness of different morphologic and immunohistochemical criteria in avoiding such diagnostic misinterpretation.

MATERIALS AND METHODS

All surgically resected carcinoid tumors, either typical or atypical, dating from 1991 to 2003, were collected from the files of the participating institutions or from the consultation files of one of the authors (J.R.) and selected for the presence

From the *Division of Pathology and Laboratory Medicine, European Institute of Oncology and University of Milan School of Medicine; and †Department of Pathology, National Cancer Institute, Milan, Italy.

Reprints: Giuseppe Pelosi, MD, Divisione di Anatomia Patologica e Medicina di Laboratorio, Istituto Europeo di Oncologia, Via G. Ripamonti, 435, I-20141 Milano, Italy (e-mail: giuseppe.pelosi@ieo.it).

of preoperative fiberoptic bronchoscopic biopsies. All cases showing a discordant diagnosis of small-cell carcinoma in the biopsy and of carcinoid tumor in the surgical specimen were selected for further evaluation. In each case, all available paraffin blocks or unstained sections were retrieved and the original hematoxylin and eosin-stained sections were reviewed. Bronchial biopsies from 9 consecutive patients with a clinically confirmed diagnosis of small-cell lung carcinoma were also retrieved from the files of the Department of Pathology of the European Institute of Oncology (Milan, Italy) and used as control group for histologic and immunohistochemical purposes. The diagnosis and typing of these tumors were carried out according to the guidelines of the 1999 WHO classification of lung and pleural tumors as they specifically apply to neuroendocrine tumors.[68]

The immunohistochemical stains were performed on formalin-fixed, paraffin-embedded tissue sections using the primary antibodies listed in Table 1 and a commercially available detection kit (EnVision Plus-HRP, Dako, Glostrup, Denmark), according to the manufacturer's suggestions. Peroxidase activity was visualized with 3-3′-diaminobenzidine-copper sulfate (Sigma Chemical Co, St. Louis, MO) to obtain a brown-black end product. The specificity of all immunoreactions was double-checked by substituting the primary antibodies with nonrelated isotypic mouse immunoglobulins at a comparable dilution, or with normal serum alone. Appropriate internal and external positive controls were also used in all immunostaining procedures to ensure specificity of reaction.

RESULTS

Clinical Features

Seven patients with a discordant histologic diagnosis of small-cell carcinoma in the biopsy (performed in either U.S. or Italian institutions in all but 1 case) and of carcinoid tumor in the surgical specimen were included in the study. The most relevant clinicopathologic and demographic data of these patients are listed in Table 2. There were four males and three females, aged from 25 to 71 years (mean ± SD, 49 ± 16 years; median, 44 years). Smoking information was available in 5 patients only, all of whom were either current or former smokers, whereas all the 9 small-cell carcinoma patients from the control group were current heavy cigarette smokers, with at least one pack/day for more than 20 years.

The indications for surgery in these 7 patients were lack of objective response to combination chemotherapy (6 patients) or radiotherapy (2 patients), or the clinical discrepancy between the initial diagnosis of small-cell carcinoma and the subsequent tumor behavior (1 patient, patient no. 3). At the time of diagnosis, the tumors presented as single pulmonary masses in all but 2 patients (patient nos. 4 and 5) in whom multiple bilateral nodules were discovered. Five patients (patient nos. 1, 2, 3, 6, and 7) underwent radical surgery, 1 (patient no. 5) underwent a wedge diagnostic excision of two peripheral nodules, and 1 (patient no. 4) had chemotherapy alone. The 5 patients for whom specific follow-up information was available were alive and well at the time of the current study in 2 cases (patient nos. 6 and 7) or alive with persistent disease in 3 cases (patient nos. 1, 2, and 5). Two patients were lost to follow-up (patient nos. 3 and 4).

Gross Features

The number of tissue fragments obtained at fiberoptic bronchoscopy ranged from 2 to 4 (mean ± SD, 3.0 ± 0.8; median, 3), and the size of biopsy tissue fragments ranged from 0.1 to 0.4 cm (mean ± SD, 0.25 ± 0.11 cm; median, 0.2 cm). None of the bronchial biopsies was accompanied by concomitant bronchial cytologic specimens. Only 1 patient (patient no. 1) underwent fine-needle aspiration cytology of a liver metastasis 2 months after performing the bronchial biopsy, with a final diagnosis of small-cell carcinoma being rendered also in these cytologic smears (original slides not available for review). In the nine small-cell carcinomas from the control group, the number and the size of biopsy tissue fragments ranged from 2 to 4 (mean ± SD, 3.2 ± 0.8; median, 3), and from 0.1 to 0.4 cm (mean ± SD, 0.24 ± 0.11 cm; median, 0.2 cm), respectively. The number and size differences between the two groups were not statistically significant.

The surgically resected carcinoid tumors ranged from 0.5 to 4.3 cm in greatest diameter. Tumors presented as either centrally located endobronchial polyps (patient no. 3) or iceberg tumor masses with bronchial involvement and intrapulmonary extension (patient nos. 1, 2, 4, 6, and 7), or as peripheral nodules with no apparent connection to the bronchial tree (patient no. 5). All excised tumors appeared grossly as well-circumscribed soft masses with hemorrhagic or glistening, pinkish-tan to graysh cut surface, with no evidence of necrosis or calcification.

Microscopic Features

Representative features of carcinoid tumors as seen in hematoxylin and eosin-stained sections of small bronchial

TABLE 1. Antibody Panel Used in the Study

Antibodies	Clone	Source	Dilution	Pretreatment
Cytokeratin pool	AE1-AE3	Novocastra Laboratories, Newcastle upon Tyne, UK	1:50	MWO-CB
Chromogranin A	LK2H10	Signet Laboratories, Dedham, MA	1:40	—
Synaptophysin	SY38	DAKO, Glostrup, Denmark	1:20	MWO-CB
Ki-67 antigen	MIB-1	DAKO, Glostrup, Denmark	1:200	MWO-EDTA
TTF-1	8G7G3/1	NeoMarkers, Union City, CA	1:50	MWO-EDTA

MWO-CB, microwave oven at 750 W for 20 minutes in citrate buffer, pH 6; MWO-EDTA, microwave oven at 750 W for 12 minutes in EDTA buffer, pH 8.

TABLE 2. Clinicopathologic Data of the 7 Pulmonary Carcinoid Patients Under Evaluation Assessed on Bronchoscopic Biopsy

Patient No.	Age (yr)/Sex	Smoking Habit	Cytologic Diagnosis	Biopsy Diagnosis	Eventual Diagnosis	Tumor Size (cm)	Surgery	Metastasis at Diagnosis	Metastasis at FU	Preoperative Therapy	DFS	OS	Clinical Outcome
1	44/M	na	SCLC	SCLC	AC	4.3	Left pneumonectomy	—	Liver	CT	2	9	AWD
2	52/M	Current	na	SCLC	AC	5.6	Left pneumonectomy	—	MLN, brain	CT	12	48	AWD
3	25/M	Former	na	SCLC	TC	1.8	Left lobectomy	—	Lost	No	na	na	Lost
4	71/M	na	na	SCLC	AC	na*	None	—	Bone, liver	CT	na	na	Lost
5	41/F	Current	na	SCLC	AC	0.8 and 0.5*	Segmentectomy, pleural biopsy	MLN, pleura	Bone	CT+RT	23	23	AWD
6	43/F	Former	na	SCLC	AC	3.3	Bilobectomy	MLN	—	CT+RT	25	25	AW
7	69/F	Current	na	SCLC	TC	2	Right lobectomy	—	—	CT	28	28	AW

DFS, disease-free survival; OS, overall survival; SCLC, small-cell lung carcinoma; AC, atypical carcinoid; TC, typical carcinoid; FU, follow-up; CT, chemotherapy; RT, radiotherapy; BSB, bronchoscopic biopsy; AWD, alive with disease; AW, alive and well; MLN, mediastinal LN; na, not available.

*These patients presented with multiple and bilateral pulmonary nodules. In the former, no surgery was tried and the patient (4) was treated with chemotherapy alone; in the latter, wedge biopsy of two peripheral nodules was carried out for diagnostic typing of the tumor.

biopsies and surgical specimens are depicted in Figures 1 to 3. Biopsy tissue fragment examination showed interlacing cords, nests, or solid aggregates of tightly packed polygonal to spindle tumor cells with scant eosinophilic cytoplasm and roundish to elongated nuclei, located beneath an intact surface bronchial epithelium showing squamous metaplasia in 2 cases (patient nos. 2 and 3). Nuclear details, which were appreciable only in the better-preserved (viable and noncrushed) areas of the tumor, were characterized by a finely granular to slightly clumped chromatin pattern, with inconspicuous to small nucleoli. More extensive crush artifacts amounting to more than 50% of the tissue fragments were seen in 4 cases (patient nos. 1–4), in the form of shrunken and barely recognizable tumor cells that could give rise to thin stromal filaments of hematoxyphilic material. Mitotic figures, tumor cell necrosis, Azzopardi's effect, and nuclear molding were not discernible in the biopsy specimens. The stroma was generally scant and highly vascularized, with thin-walled and variably dilated blood vessels intermingled to and separating small tumor cell aggregates (Table 3).

Microscopic examination of the surgically excised lung specimens showed the features of typical carcinoid tumor (up to 1 mitosis/10 HPF, no necrosis) in 2 cases (patient nos. 3 and 7), and or atypical carcinoid tumor (1 to 4 mitoses/10 HPF, punctate necrosis) in the other 5 cases (patient nos. 1, 2, 4, 5, and 6).

By contrast, the small-cell carcinoma biopsies from the control cases exhibited solid aggregates of oval to spindle tumor cells with hyperchromatic and molding nuclei, high nucleo/cytoplasmic ratio, evenly dispersed chromatin, inconspicuous nucleoli, moderate to extensive geographic necrosis, and crushing artifacts with stromal linear basophilia (Figs. 3 A_2, 4A). Squamous cell metaplasia of the bronchial epithelium was seen in 2 cases (SCLC 6, 7) (Fig. 4B), whereas the Azzopardi's phenomenon was not detectable in the histologic material under evaluation. Mitotic figures were plentiful (Table 4). The stroma was generally fibrotic and contained proliferating vessels with glomeruloid configuration or long cords of thick-walled vessels (Fig. 4A).

Immunohistochemical Features

Representative features of immunostains in bronchial small biopsy fragments of carcinoid tumor and small-cell carcinoma are depicted in Figure 3B–E. Chromogranin A and synaptophysin immunoreactivity, which was assessed in all but 1 case (patient no. 1), appeared as either a granular or diffuse labeling of the cytoplasm in 10% to >50% of the tumor cells. Cytokeratin immunoreactivity, which was evaluable in all but 1 case (patient no. 6), was present in the form of either diffuse cytoplasmic or dot-like paranuclear accumulation of the immunostaining product. TTF-1 immunoreactivity was not seen in any of the tested biopsies. Proliferative activity, as assessed by Ki-67 labeling index, ranged from 1% to 17% of the tumor cells (mean ± SD, 4.6 ± 6.1; median, 2). All these immunostaining patterns were usually detectable also in crushed tumor areas, and this was particularly true for Ki-67. However, it was sometimes difficult to discern decoration for

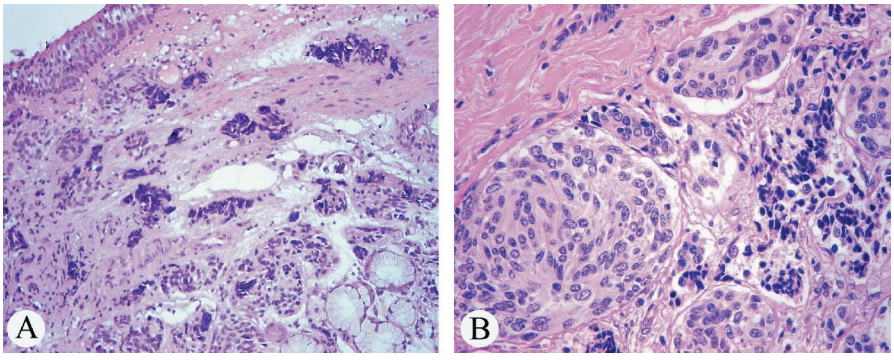

FIGURE 1. Histologic features of carcinoid tumor (case no. 2) showing crush artifacts of tumor cells both in the small tissue biopsy (A) and in the surgical specimen (B). Remnants of intact bronchial epithelium undermined by better preserved tumor cells and bronchial mucous glands surrounded by viable neoplastic cells are visible in the upper left and lower right corners of the specimen, respectively (A).

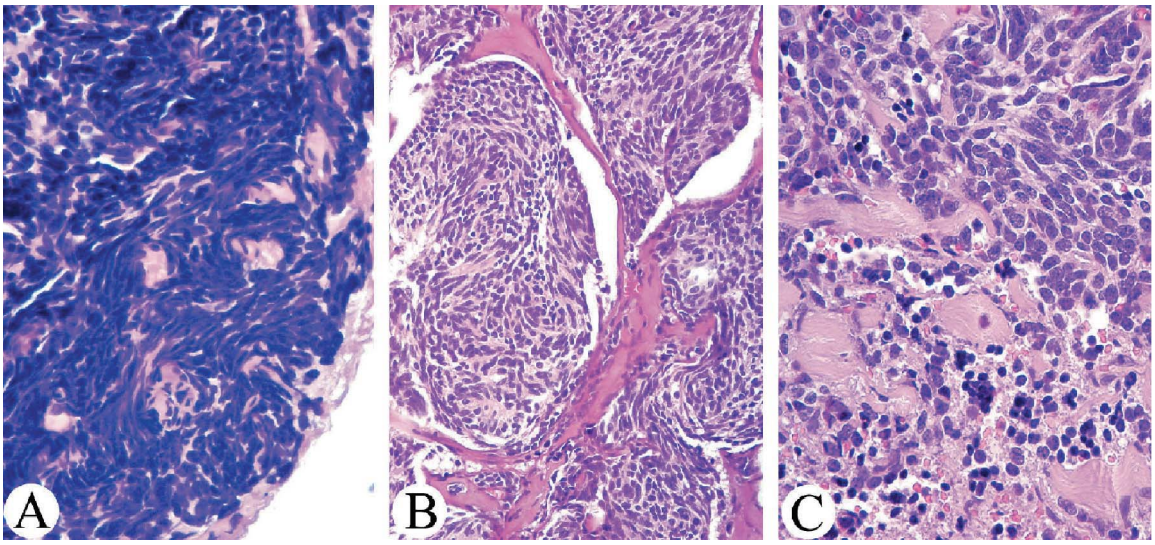

FIGURE 2. Carcinoid tumor (case no. 1) showing severe crush artifacts of tumor cells in the biopsy specimen (A), resulting in a striking resemblance to small-cell carcinoma. The surgical specimen revealed a tumor composed of spindle cells arranged in short fasicles and whorls (B) that focally exhibited crush artifacts (C).

cytoplasmic markers in the most severely damaged areas. Comparable immunoreactivity patterns were observed in the resected carcinoid tumor specimens, and no significant differences were observed in terms of both the number or intensity of immunoreactive cells and the pattern of immunostaining. Ki-67 labeling index in surgical specimens ranged from 6% to 23% of tumor cells (mean ± SD, 10.6 ± 7.1; median, 8.2), but this difference was not statistically significant (Table 3).

By contrast, the biopsies from the control group of small-cell carcinomas showed a noticeable reduction of chromogranin A and synapthophysin immunostaining, whereas a focal to diffuse dot-like paranuclear decoration for cytokeratins was maintained in most cases. Diffuse TTF-1 immunoreactivity was recognizable in 5 cases (SCLC 1, 2, 3, 5, and 8), focal in one (SCLC 4), and absent in three (SCLC 6, 7, and 9). The Ki-67 labeling index ranged from 60% to 96% of tumor cells (mean ± SD, 81.2 ± 13.4; median, 85). These patterns of immunoreactivity were generally recognizable also in areas with crush artifacts, although it was sometimes difficult to discern decoration for cytoplasmic markers in the most severely damaged areas.

DISCUSSION

The result of our study show that the overdiagnosis of pulmonary carcinoid tumor as small-cell carcinoma is a rare but real occurrence with potentially dangerous consequences because of the choice of therapy that can be instituted as a result. The standard treatment of carcinoid tumors, whether typical or atypical, is complete surgical resection, whereas the role of radio-chemotherapy in a multimodality treatment mode or for paliation for these tumors remains controversial.[27,73] The distinctions between small-cell and large-cell neuroendocrine carcinoma, between typical and atypical carcinoid tumor, and between and atypical carcinoid tumor and large-cell neuroendocrine carcinomas remain highly controversial and frequently discussed issues in the field of pulmonary neuroendocrine tumors,[68–70] whereas the fact that carcinoid tumor may be overinterpreted as small-cell carcinoma is rarely mentioned.

Several histologic criteria have been proposed in the 1999 WHO classification for distinguishing pulmonary carcinoid tumors, either typical or atypical, from small-cell carcinomas.[3,14,51,68] Alas, these features are best appreciated on correctly handled and optimally fixed surgical specimens, whereas the examination of small biopsy fragments may prove to be considerably more challenging.

Demographic and clinical information is of no great use in this regard.[72] Although the peak age incidence is reportedly lower in typical and atypical carcinoid tumors than in small-cell carcinomas, our series confirms the well-known fact that carcinoid tumors may occasionally occur in elderly patients (patient nos. 4 and 7) and that small-cell carcinomas may be seen in younger patients (SCC-2, 4, and 6) (Table 4). Smoking habit information and gender prevalence are even less informative. This is especially true for atypical carcinoid tumors (the ones most likely to be overinterpreted),[72] in the sense that, like small-cell carcinomas, they are most often seen in male smokers.[14,48] A more useful parameter is tumor location, as atypical carcinoid tumors are usually situated at the lung periphery, unlike small-cell carcinomas.[46,55] Parenthetically, this was not true in our series, in the sense that most of the tumors presented as iceberg lesions (patient nos. 1, 2, 4, and 6), with only 1 case being peripheral (patient no. 5). As far as typical carcinoid tumors are concerned, they usually arise as endobronchial polyps or iceberg masses,[32,48] as our series once again demonstrated (patient nos. 3 and 7), whereas most small-cell carcinomas give rise to bulky involvement and narrowing of major airway walls, with only exceptional instances being reported as bronchus-occluding polypoid growths.[13,30]

The number and size of the biopsy samples did not influence the likelihood of correct diagnosis in our series,

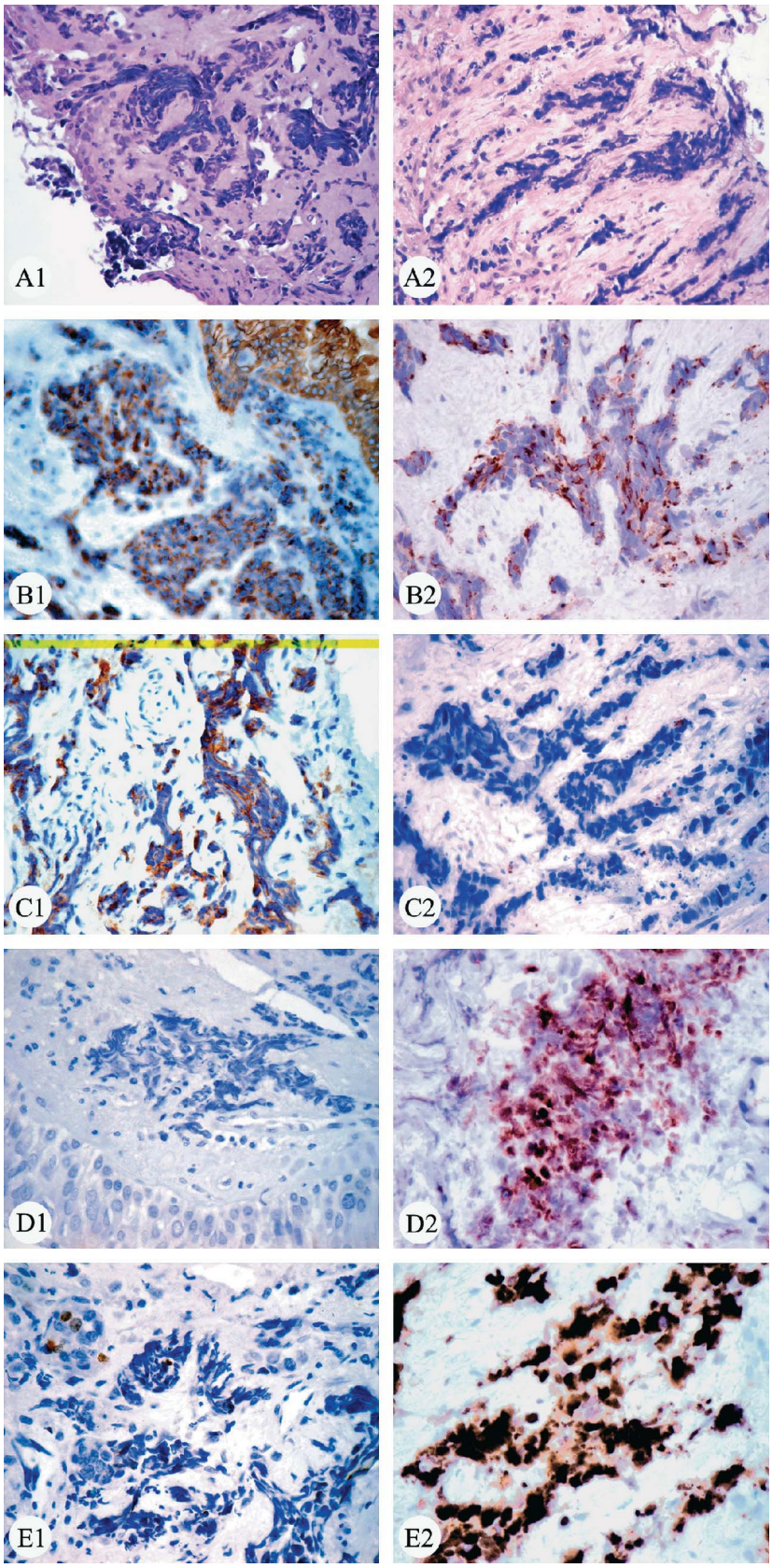

FIGURE 3. Comparative evaluation of carcinoid tumor (case no. 2) and small-cell carcinoma (case no. 3) in small tissue biopsy fragments. At the hematoxylin and eosin level, the two tumor types are virtually undistinguishable in severely crushed areas, in which the fine evaluation of the chromatin pattern of tumor cells is not feasible ($A_{1\text{-}2}$). Immunohistochemically, cytokeratins may show a similar dot-like staining pattern ($B_{1\text{-}2}$). Chromogranin A reactivity is usually greater in carcinoid tumor ($C_{1\text{-}2}$), whereas TTF-1 ($D_{1\text{-}2}$) staining (in this case, a surgical specimen) and, particularly, Ki-67 (MIB-1) labeling index ($E_{1\text{-}2}$) are more prominent in small-cell carcinoma.

TABLE 3. Pathologic and Immunohistochemical Results on the Series of Seven Carcinoid Tumors

Patient No.	Histologic Diagnosis	Squamous Metaplsia	Cell Necrosis		Crush Artifacts	Azzopardi Effect	Mitosis/10HPF		CK		CgA		Syn		TTF-1		Ki-67 (%)	
			Bio	SS			Bio	SS	Bio	SS	Bio	SS	Bio	SS	Bio	SS	Bio	SS
1	AC	No	No	+/−	+++	No	0	1	++^{cyt}	++^{cyt}	+++	+++	na	+++	−	−	1	9.8
2	AC	Yes	No	+/−	+++	No	0	4	++^{cyt}	++^{cyt}	+++	+++	+++	+++	−	−	8	8.2
3	TC	Yes	No	No	+++	No	0	0	+++^{cyt}	na	+++	na	+	na	−	na	1	na
4	AC	No	No	+/−	+++	No	0	3	+/−^{cyt}	na	+++	na	+++	na	−	na	3	na
5	AC	No	No	+/−	++	No	0	2	+/−^{cyt}	−	+	+++	+++	+++	−	−	17	23
6	AC	No	No	+/−	+/−	No	0	1	na	+/−^{cyt}	+++	+++	+++	+++	na	++	1	6
7	TC	No	No	No	+/−	No	0	1	+++^{dot}	+++^{dot}	+++	+++	+++	+++	−	−	1	5.8

CK, cytokeratin pool; CgA, chromogranin A; Syn, synaptophysin; Bio, biopsy; SS, surgical specimen; cyt, cytoplasmic staining; dot, paranuclear, dot-like immunostaining; +/−, <10%; +, 10–25%; ++, 26–50%; +++, >50%.

provided that a sufficient amount of well-preserved tissue was available for pathologic evaluation.

Artifacts in lung tumor tissue due to the operative procedure, delayed fixation, or poor processing are major causes of incorrect diagnostic interpretations.[36] Improper handling, fixation, and processing of biopsies may induce nuclear chromatin condensation and shrinking, poor preservation of cytologic details, and extracellular displacement of hematoxyphilic material with strands and masses into the stroma,[12,14] increasing the risk of misclassification. A careful histologic evaluation of the viable, well-preserved, and nontraumatized portion of the tumor, however scanty that portion may be, is important to prevent misclassification.

The key factor for correctly distinguishing small-cell carcinomas from carcinoid tumors remains an accurate evaluation of the nuclear features as seen in hematoxylin and eosin-stained sections. The finely and evenly dispersed chromatin with inconspicuous or undetectable nucleoli of small-cell carcinoma cells contrasts with the finely granular chromatin of typical carcinoid tumors and with the more clumped chromatin in a vesicular nucleus with variably sized nucleolus of atypical carcinoid tumors.[68] These nuclear features can be well appreciated even in cytologic prepations from sputum, bronchial secretions or washings, or aspiration biopsy,[21,24,34,37,40,43,44,63,64] once the hurdle of initial recognition of small cancer cells has been overcome, especially by inexperienced observers.[38] A practical conclusion, however, is that, regardless of cytologic diagnosis, every small lung lesion should be excised and examined histologically, so long as the diagnosis is not that of small-cell carcinoma.[38]

Moderate to severe crushing of tumor cells was detected in five carcinoids of our series, with displacement of chromatin debris into the stroma but never showing the features of hematoxyphilic incrustation of the vessel walls (so-called Azzopardi's effect or sign).[6] On the other hand, crush artifact can also be seen with lymphoid lesions and peripheral neuroectodermal tumors, the latter being notably rare in the lung.[71] In these cases, however, appropriate immunohistochemistry, including lymphoid markers and CD99,[71] may assist in the diagnosis. Although none of the biopsies of our small-cell carcinoma series exhibited the Azzopardi sign (probably because of the small size of the tissue fragments), the identification of this effect in a biopsy of neuroendocrine lung tumor virtually excludes carcinoid tumor.[13] However, it is worth mentioning that the Azzopardi effect is not pathognomonic of small-cell carcinoma, since other pulmonary malignancies or even inflammatory infiltrates may give rise to a similar alteration.[13,14] None of the misinterpreted carcinoid tumors of the current series showed tumor necrosis that was instead detectable in all small cell-carcinomas of the control group. Lack of tumor necrosis, however, does not allow a reliable subtyping of the tumor if crushing artifacts or poor preservation hampers the fine evaluation of tumor cell morphology. Furthermore, necrosis per se is not diagnostic of small-cell carcinoma, since it may also occur, sometimes in an extensive fashion, in atypical carcinoid tumors.[13,14,68] Similar consideration holds true for squamous cell metaplasia, which was as prevalent in carcinoid tumors (patient nos. 2 and 3) as in small-cell carcinomas (SCLC 6, 7). An interesting diagnostic clue was the different pattern of vascularization as seen in

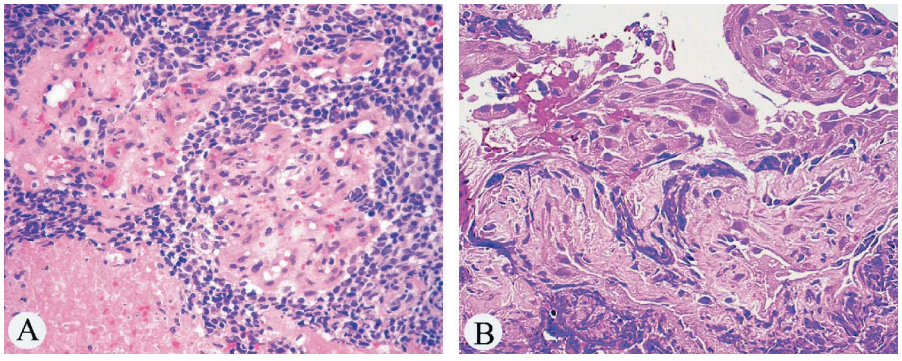

FIGURE 4. Small-cell carcinoma (case no. 4) showing highly proliferating blood vessels with a glomeruloid configuration. The tumor cells have a finely and evenly dispersed chromatin pattern and exhibit areas of coagulative necrosis (left lower corner) (A). Another example of small-cell carcinoma (case no. 6) featuring foci of squamous cell metaplasia in the surface bronchial epithelium, which in turn is undermined by tumor cells in the lamina propria (bottom) (B).

TABLE 4. Pathologic and Immunohistochemical Results on the Series of Nine Small-Cell Carcinomas

Patient	Age (yr)/Sex	Squamous Metaplsia	Cell Necrosis	Crush Artifacts	Azzopardi Effect	Mitosis/ 10 HPF	CK	CgA	Syn	TTF-1	Ki-67 (%)
SCLC 1	64/M	No	++	+++	No	12	$+++^{dot}$	+/−	++	+++	96
SCLC 2	47/M	No	+++	+++	No	15	$+++^{dot}$	+	+	+++	85
SCLC 3	81/F	No	++	+	No	18	$+++^{dot}$	++	+	+++	88
SCLC 4	56/M	No	++	++	No	11	$+++^{dot}$	neg	neg	+/−	95
SCLC 5	70/F	No	+++	+	No	11	$++^{dot}$	+/−	+/−	+++	85
SCLC 6	51/M	Yes	++	+++	No	12	$++^{dot}$	neg	++	neg	92
SCLC 7	63/M	Yes	++	+++	No	15	$+^{dot}$	+/−	++	neg	65
SCLC 8	75/M	No	++	++	No	11	$+++^{dot}$	+/−	+/−	+++	60
SCLC 9	63/M	No	++	+++	No	12	$++^{dot}$	neg	+	neg	70

CK, cytokeratin pool; CgA, chromogranin A; Syn, synaptophysin; dot, paranuclear, dot-like immunoreactivity product; +/−, <10% of tumor cells; +, 10–25%; ++, 26–50%; +++, >50%.

hematoxylin and eosin-stained sections. Carcinoid tumors showed thin-walled and variably dilated blood vessels, whereas small-cell carcinomas exhibited highly proliferating vessels with glomeruloid configuration or long cords of thick-walled vascular channels. This is likely to be due to the production of angiogenic factors by carcinoma cells, as previously indicated both in brain metastases from pulmonary small-cell carcinomas[33] and in other types of high-grade neural and neuroendocrine primary neoplasms.[22]

Immunohistochemical evaluation may contribute to the correct classification of pulmonary neuroendocrine tumors.[5,14] Cromogranin A and synaptophysin are currently regarded as providing the best compromise in terms of sensitivity and specificity for the identification of neuroendocrine features in tumor samples.[9,23] As reported by others, we found higher levels of immunoreactivity for these cytoplasmic markers in carcinoid tumors than in small-cell carcinomas,[7,26,67] in keeping with the statement made by several authors that neuroendocrine tumors expressing high levels of chromogranin A and synaptophysin are unlikely to be poorly differentiated neuroendocrine carcinomas, whether in the lung[7,67] or in extrapulmonary anatomic sites.[29] Conversely, cytokeratin immunoreactivity is not contributory to the diagnosis, inasmuch as carcinoid tumor and small-cell carcinoma may share low levels of immunostaining and a paranuclear dot-like distribution of this marker both in bronchial biopsies and open-lung specimens. Several other markers have been reported to be differentially expressed in carcinoid tumors and small-cell lung carcinomas, including the polysialic form of CD56/NCAM,[42], p53,[7,54] an altered bcl-2:bax ratio,[10] and an altered p16:retinoblastoma pathway,[15] but the staining patterns are not different enough to reliably classify individual tumors. The assessment of the proliferative fraction by Ki-67 immunostaining is much more useful in this regard,[2,4,41,57,59] being that small-cell carcinomas are characterized by a proliferative fraction exceedingly higher than typical and atypical carcinoid tumor, as confirmed in our series. Furthermore, we have recently investigated 220 surgically resected neuroendocrine tumors of the lung for this marker and showed that the median value of Ki-67 immunoreactivity was 2.3% in 100 typical carcinoids, 9% in 36 atypical carcinoids, 47.5% in 52 large-cell neuroendocrine carcinomas, and 64.5% in 32 small-cell carcinomas (Pelosi G, unpublished observations).

TTF-1 immunoreactivity has been seen to occur with a variable frequency[11,16,19,35,53] or to be absent[17,62] in typical and atypical pulmonary carcinoid tumors, while is consistently expressed in most small-cell carcinomas, either pulmonary[19,35] or extra-pulmonary.[1,35] In our series, none of the carcinoids as evaluated on small biopsy tissue fragment and only one (patient no. 6) of those evaluated on surgical specimens showed TTF-1 immunoreactivity, whereas most small-cell carcinomas in the control group exhibited focal to diffuse nuclear decoration of the tumor cells.

The immunostaining for cytoplasmic markers, including cytokeratins, chromogranin A and synaptophysin, was relatively well preserved in the crushed areas of both carcinoid

TABLE 5. Relevant Differences Between Carcinoid Tumor and Small-Cell Carcinoma on Biopsy Tissue Fragments

Feature	Carcinoid Tumor	Small-Cell Carcinoma
Tumor location	Central or peripheral	Central (less usually peripheral)
Chromatin pattern	Granular, sometimes coarse	Finely and evenly dispersed
Blood vessels	Thin-walled	Thick-walled, glomeruloid pattern
Mitoses	≤10 mitoses/10 HPF or 2 mm^2	>10 mitoses/10 HPF or 2 mm^2
Chromogranin A and synaptophysin	Higher level of immunoreactivity	Lower level of immunoreactivity
Ki-67 (MIB-1) labeling index	Low to moderate (typically <20%)	High (typically >50%)

HPF, high power fields, by using a microscope with a 40× objective, an eyepiece field-of-view number of 20, and no magnification changing devices.

tumors and small-cell carcinomas, although it was sometimes difficult to discern residual decoration in the most severely damaged areas. On the contrary, the immunoreactivity for nuclear markers (especially Ki-67) was well preserved and easy to be assessed even in the areas with extensive crushing artifacts of both tumor types, thus providing an important and effective diagnostic clue in this situation. The most helpful criteria in distinguishing carcinoid tumors from small-cell carcinomas on small biopsy tissue fragments are listed in Table 5. By applying these criteria, we confirmed the initial diagnosis of small-cell carcinoma on bronchial biopsies also in long-surviving patients with limited-disease small-cell carcinoma treated with chemotherapy only (data not shown).

In summary, this series shows that the possibility of overdiagnosing carcinoid tumor as small-cell carcinoma remains a real one in a worldwide sample of hospital settings. A constellation of clinical, morphologic, and immunohistochemical data should be taken into account to reach a correct diagnosis. Careful evaluation of the hematoxylin and eosin sections remains the most important tool in this differential diagnosis, with evaluation of tumor cell proliferation by Ki-67 labeling index emerging as the most useful ancillary technique. Other immunohistochemical reactions may be used for confirming the epithelial or the neuroendocrine differentiation of the neoplasm, but are less likely to assist in this specific differential diagnosis.

REFERENCES

1. Agoff SN, Lamps LW, Philip AT, et al. Thyroid transcription factor-1 is expressed in extrapulmonary small cell carcinomas but not in other extrapulmonary neuroendocrine tumors. *Mod Pathol*. 2000;13:238–242.
2. Arbiser ZK, Arbiser JL, Cohen C, et al. Neuroendocrine lung tumors: grade correlates with proliferation but not angiogenesis. *Mod Pathol*. 2001;14:1195–1199.
3. Arora VK, Singh N, Chaturvedi S, et al. Significance of cytologic criteria in distinguishing small cell from non-small cell carcinoma of the lung. *Acta Cytol*. 2003;47:216–220.
4. Aslan DL, Gulbahce HE, Pambuccian SE, et al. Preservation of immunoreactivity for MIB-1 aids in the differential diagnosis of pulmonary small cell carcinoma and carcinoid tumor in specimens with extensive crush artifacts [Abstract 1403]. *Mod Pathol*. 2004;17(suppl 1):333.
5. Axiotis CA. The neuroendocrine lung. In: Li Volsi V, Asa SL, eds. *Endocrine Pathology.* New York: Churchill Livingstone, 2002:261–296.
6. Azzopardi JG. Oat-cell carcinoma of the bronchus. *J Pathol Bacteriol*. 1959;78:513–519.
7. Barbareschi M, Girlando S, Mauri FA, et al. Tumour suppressor gene products, proliferation, and differentiation markers in lung neuroendocrine neoplasms. *J Pathol*. 1992;166:343–350.
8. Bean DM, Haldenby DA. Endobronchial adenomata in children. *J Otolaryngol*. 1976;5:519–522.
9. Brambilla E, Lantuejoul S, Sturm N. Divergent differentiation in neuroendocrine lung tumors. *Semin Diagn Pathol*. 2000;17:138–148.
10. Brambilla E, Negoescu A, Gazzeri S, et al. Apoptosis-related factors p53, Bcl2, and Bax in neuroendocrine lung tumors. *Am J Pathol*. 1996;149: 1941–1952.
11. Cai YC, Banner B, Glickman J, et al. Cytokeratin 7 and 20 and thyroid transcription factor 1 can help distinguish pulmonary from gastrointestinal carcinoid and pancreatic endocrine tumors. *Hum Pathol*. 2001;32:1087–1093.
12. Choi H, Byhardt RW, Clowry LJ, et al. The prognostic significance of histologic subtyping in small cell carcinoma of the lung. *Am J Clin Oncol*. 1984;7:389–397.
13. Colby TV, Koss MN, Travis WD. *Tumors of the Lower Respiratory Tract*. Washington, DC: Armed Forces Institute of Pathology, 1995.
14. Corrin B. *Pathology of the Lungs*. London: Churchill Livingstone, 2000.
15. Dosaka-Akita H, Cagle PT, Hiroumi H, et al. Differential retinoblastoma and p16(INK4A) protein expression in neuroendocrine tumors of the lung. *Cancer*. 2000;88:550–556.
16. Du EZ, Goldstraw P, Zacharias J, et al. TTF-1 expression is specific for lung primary in typical and atypical carcinoids: TTF-1-positive carcinoids are predominantly in peripheral location. *Hum Pathol*. 2004;35:825–831.
17. Fabbro D, Di Loreto C, Stamerra O, et al. TTF-1 gene expression in human lung tumours. *Eur J Cancer*. 1996;32:512–517.
18. Flieder DB. Neuroendocrine tumors of the lung: recent developments in histopathology. *Curr Opin Pulm Med*. 2002;8:275–280.
19. Folpe AL, Gown AM, Lamps LW, et al. Thyroid transcription factor-1: immunohistochemical evaluation in pulmonary neuroendocrine tumors. *Mod Pathol*. 1999;12:5–8.
20. Forster BB, Muller NL, Miller RR, et al. Neuroendocrine carcinomas of the lung: clinical, radiologic, and pathologic correlation. *Radiology*. 1989; 170:441–445.
21. Frierson HF Jr, Covell JL, Mills SE. Fine needle aspiration cytology of atypical carcinoid of the lung. *Acta Cytol*. 1987;31:471–475.
22. Gaudin PB, Rosai J. Florid vascular proliferation associated with neural and neuroendocrine neoplasms: a diagnostic clue and potential pitfall. *Am J Surg Pathol*. 1995;19:642–652.
23. Gosney JR, Gosney MA, Lye M, et al. Reliability of commercially available immunocytochemical markers for identification of neuroendocrine differentiation in bronchoscopic biopsies of bronchial carcinoma. *Thorax*. 1995;50:116–120.
24. Gottlieb C, Kini S, Feingold M. Cytomorphometric analysis of small cell neoplasms of the lung from specimens obtained via bronchoscopy. *Anal Quant Cytol Histol*. 1992;14:41–46.
25. Gripp FM, Risse EK, Leverstein H, et al. Neuroendocrine neoplasms of the larynx: importance of the correct diagnosis and differences between atypical carcinoid tumors and small-cell neuroendocrine carcinoma. *Eur Arch Otorhinolaryngol*. 1995;252:280–286.
26. Guinee DG Jr, Fishback NF, Koss MN, et al. The spectrum of immunohistochemical staining of small-cell lung carcinoma in specimens from transbronchial and open-lung biopsies. *Am J Clin Pathol*. 1994;102:406–414.
27. Hage R, de la Riviere AB, Seldenrijk CA, et al. Update in pulmonary carcinoid tumors: a review article. *Ann Surg Oncol*. 2003;10: 697–704.
28. Hamperl H. Über gutartige Bronchialtumoren (Cylindrome und Carcinoide). *Virchows Arch Pathol Anat*. 1937;300:46–88.
29. Helpap B, Kollermann J. Immunohistochemical analysis of the proliferative activity of neuroendocrine tumors from various organs: are there indications for a neuroendocrine tumor-carcinoma sequence? *Virchows Arch*. 2001;438:86–91.
30. Horio H, Nomori H, Origasa H, et al. Early small cell lung cancer with extensive inflammation and scar formation. *Jpn J Thorac Cardiovasc Surg*. 2000;48:824–826.
31. Hurt R, Bates M. Carcinoid tumours of the bronchus: a 33 year experience. *Thorax*. 1984;39:617–623.
32. Ishida T, Yokoyama H, Sugio K, et al. Carcinoid tumor of the lung: clinicopathological and immunohistochemical studies. *Eur J Surg Oncol*. 1992;18:180–187.
33. Ito T, Kitamura H, Nakamura N, et al. A comparative study of vascular proliferation in brain metastasis of lung carcinomas. *Virchows Arch A Pathol Anat Histopathol*. 1993;423:13–17.
34. Jordan AG, Predmore L, Sullivan MM, et al. The cytodiagnosis of well-differentiated neuroendocrine carcinoma. A distinct clinicopathologic entity. *Acta Cytol*. 1987;31:464–470.
35. Kaufmann O, Dietel M. Expression of thyroid transcription factor-1 in pulmonary and extrapulmonary small cell carcinomas and other neuroendocrine carcinomas of various primary sites. *Histopathology.* 2000;36:415–420.
36. Kendall DM, Gal AA. Interpretation of tissue artifacts in transbronchial lung biopsy specimens. *Ann Diagn Pathol*. 2003;7:20–24.
37. Kim K, Mah C, Dominquez J. Carcinoid tumors of the lung: cytologic differential diagnosis in fine-needle aspirates. *Diagn Cytopathol*. 1986;2:343–346.
38. Koss LG. Tumors of the lung: conventional cytology and aspiration biopsy. In: Koss LG, ed. *Diagnostic Cytology and Its Histopathologic Bases*. Philadelphia: Lippincott, 1992:769–864.

39. Kron IL, Harman PK, Mills SE, et al. A reappraisal of limited-stage undifferentiated carcinoma of the lung: does stage I small cell undifferentiated carcinoma exist? *J Thorac Cardiovasc Surg*. 1982;84:734–747.
40. Kyriakos M, Rockoff SD. Brush biopsy of bronchial carcinoid: a source of cytologic error. *Acta Cytol*. 1972;16:261–268.
41. Laitinen KL, Soini Y, Mattila J, et al. Atypical bronchopulmonary carcinoids show a tendency toward increased apoptotic and proliferative activity. *Cancer*. 2000;88:1590–1598.
42. Lantuejoul S, Moro D, Michalides RJ, et al. Neural cell adhesion molecules (NCAM) and NCAM-PSA expression in neuroendocrine lung tumors. *Am J Surg Pathol*. 1998;22:1267–1276.
43. Leonhardt P, Gosse H. Value of cytologic diagnosis of pulmonary carcinoid tumors: a morphologico-clinical analysis. *Zentralbl Allg Pathol*. 1987;133:285–292.
44. Lozowski W, Hajdu SI, Melamed MR. Cytomorphology of carcinoid tumors. *Acta Cytol*. 1979;23:360–365.
45. Marchevsky AM, Chuang MT, Teirstein AS, et al. Problems in the diagnosis of small cell carcinoma of the lungs by fiberoptic bronchoscopy. *Cancer Detect Prev*. 1984;7:253–260.
46. Mark EJ, Ramirez JF. Peripheral small-cell carcinoma of the lung resembling carcinoid tumor: a clinical and pathologic study of 14 cases. *Arch Pathol Lab Med*. 1985;109:263–269.
47. Markel SF, Abell MR, Haight C, et al. Neoplasms of bronchus commonly designated as adenomas. *Cancer*. 1964;17:590–608.
48. McCaughan BC, Martini N, Bains MS. Bronchial carcinoids: review of 124 cases. *J Thorac Cardiovasc Surg*. 1985;89:8–17.
49. McMullan DM, Wood DE. Pulmonary carcinoid tumors. *Semin Thorac Cardiovasc Surg*. 2003;15:289–300.
50. Naidich DP, McCauley DI, Siegelman SS. Computed tomography of bronchial adenomas. *J Comput Assist Tomogr*. 1982;6:725–732.
51. Nicholson SA, Beasley MB, Brambilla E, et al. Small cell lung carcinoma (SCLC): a clinicopathologic study of 100 cases with surgical specimens. *Am J Surg Pathol*. 2002;26:1184–1197.
52. Nicholson SA, Ryan MR. A review of cytologic findings in neuroendocrine carcinomas including carcinoid tumors with histologic correlation. *Cancer*. 2000;90:148–161.
53. Oliveira AM, Tazelaar HD, Myers JL, et al. Thyroid transcription factor-1 distinguishes metastatic pulmonary from well-differentiated neuroendocrine tumors of other sites. *Am J Surg Pathol*. 2001;25:815–819.
54. Przygodzki RM, Finkelstein SD, Langer JC, et al. Analysis of p53, K-ras-2, and C-raf-1 in pulmonary neuroendocrine tumors: correlation with histological subtype and clinical outcome. *Am J Pathol*. 1996;148:1531–1541.
55. Quoix E, Fraser R, Wolkove N, et al. Small cell lung cancer presenting as a solitary pulmonary nodule. *Cancer*. 1990;66:577–582.
56. Saijo N. Progress in treatment of small-cell lung cancer: role of CPT-11. *Br J Cancer*. 2003;89:2178–2183.
57. Sampietro G, Tomasic G, Collini P, et al. Gene product immunophenotyping of neuroendocrine lung tumors: no linking evidence between carcinoids and small-cell lung carcinomas suggested by multivariate statistical analysis. *Appl Immunohistochem Mol Morphol*. 2000;8:49–56.
58. Sandler AB. Chemotherapy for small cell lung cancer. *Semin Oncol*. 2003;30:9–25.
59. Santinelli A, Ranaldi R, Baccarini M, et al. Ploidy, proliferative activity, p53 and bcl-2 expression in bronchopulmonary carcinoids: relationship with prognosis. *Pathol Res Pract*. 1999;195:467–474.
60. Smit EF, Groen HJ, Timens W, et al. Surgical resection for small cell carcinoma of the lung: a retrospective study. *Thorax*. 1994;49:20–22.
61. Spira A, Ettinger DS. Multidisciplinary management of lung cancer. *N Engl J Med*. 2004;350:379–392.
62. Sturm N, Rossi G, Lantuejoul S, et al. Expression of thyroid transcription factor-1 in the spectrum of neuroendocrine cell lung proliferations with special interest in carcinoids. *Hum Pathol*. 2002;33:175–182.
63. Suen KC, Quenville NF. Fine needle aspiration cytology of uncommon thoracic lesions. *Am J Clin Pathol*. 1981;75:803–809.
64. Szyfelbein WM, Ross JS. Carcinoids, atypical carcinoids, and small-cell carcinomas of the lung: differential diagnosis of fine-needle aspiration biopsy specimens. *Diagn Cytopathol*. 1988;4:1–8.
65. Thivolet-Bejui F. Diagnostic pitfalls in bronchopulmonary tumors. *Arch Anat Cytol Pathol*. 1997;45:249–253.
66. Torre M, Barberis M, Barbieri B, et al. Typical and atypical bronchial carcinoids. *Respir Med*. 1989;83:305–308.
67. Totsch M, Muller LC, Hittmair A, et al. Immunohistochemical demonstration of chromogranins A and B in neuroendocrine tumors of the lung. *Hum Pathol*. 1992;23:312–316.
68. Travis WD, Colby TV, Corrin B, et al. *Histological Typing of Lung and Pleural Tumours*. Berlin: Springer Verlag, 1999.
69. Travis WD, Gal AA, Colby TV, et al. Reproducibility of neuroendocrine lung tumor classification. *Hum Pathol*. 1998;29:272–279.
70. Travis WD, Rush W, Flieder DB, et al. Survival analysis of 200 pulmonary neuroendocrine tumors with clarification of criteria for atypical carcinoid and its separation from typical carcinoid. *Am J Surg Pathol*. 1998;22:934–944.
71. Tsuji S, Hisaoka M, Morimitsu Y, et al. Peripheral primitive neuroectodermal tumour of the lung: report of two cases. *Histopathology*. 1998;33:369–374.
72. Valli M, Fabris GA, Dewar A, et al. Atypical carcinoid tumour of the lung: a study of 33 cases with prognostic features. *Histopathology*. 1994;24:363–369.
73. Wirth LJ, Carter MR, Janne PA, et al. Outcome of patients with pulmonary carcinoid tumors receiving chemotherapy or chemoradiotherapy. *Lung Cancer*. 2004;44:213–220.
74. Wright ES, Pike E, Couves CM. Unusual tumours of the lung. *J Surg Oncol*. 1983;24:23–29.
75. Yoshida J, Nagai K, Takahashi K, et al. A rare case of typical carcinoid of the lung coincident with thymoma. *Jpn J Clin Oncol*. 1994;24:289–293.
76. Zych J, Slodkowska J, Burakowska B. Recurrent atypical bronchial tumor diagnosed as small cell lung cancer. *Pneumonol Alergol Pol*. 1995;63:72–76.