

A Clinicopathologic and Immunohistochemical Study of 35 Anaplastic Carcinomas of the Pancreas With a Review of the Literature

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Anaplastic pancreatic carcinomas are rare tumors, frequently displaying a variety of growth patterns. The literature lacks a comprehensive study of this tumor. Thirty-five cases of anaplastic carcinoma of the pancreas diagnosed between 1955 and 1997 were retrieved from the Endocrine Registry at the Armed Forces Institute of Pathology. Histology, immunophenotype, molecular analysis, and patient follow-up were analyzed. The tumors of 10 women and 25 men, aged 34 to 85 years (mean age at presentation, 62.5 years), were studied. Patients had vague symptoms (weight loss, pain, and fatigue, nausea, or vomiting), lasting an average of 13.2 weeks. The tumors, of an average size of 9.2 cm, were usually in the head or tail of the pancreas. The tumors were widely infiltrative, histomorphologically separated into predominantly large, pleomorphic cell, or spindle cell groups. Tumor phagocytosis and necrosis were noted. Immunohistochemical studies confirmed an epithelial origin with at least one epithelial marker in 78% of the tumors. *K-ras* mutations by sequence analysis were found in eight of 12 cases tested. Surgical biopsy/excision was used in all patients. Twenty-nine of 35 patients died of disease (average, 5.2 months), three died with no evidence of disease (average, 56.9 months), and three patients were alive at last follow-up (average, 94.0 months), one with residual disease. There was no statistically significant difference in survival between patients with and without a *K-ras* mutation. Anaplastic carcinoma of the pancreas usually occurs in the head of the pancreas in older men. The epithelial nature of the pleomorphic cells (giant or spindled) can usually be documented. Patients with *K-ras* mutations have a shorter survival time, even though the overall prognosis for all anaplastic carcinomas is fatal (93% fatality; average survival, 448 days). *Ann Diagn Pathol* 5: 129-140, 2001. This is a US government work. There are no restrictions on its use.

Index Words: Anaplastic carcinoma, pancreas, giant cell carcinoma, prognosis

THE MAJORITY of malignant tumors of the pancreas are of epithelial derivation (including endocrine carcinomas). Sarcomas and secondary tumors account for the remaining minor-

ity. Undifferentiated malignancies are generally accepted to represent probable carcinomas, making up a small percentage of all pancreatic tumors (2% to 7%).¹⁻⁶ A variety of different subtypes of anaplastic carcinoma have been described,⁷ including spindle cell, giant cell, pleomorphic giant cell, and round cell. A complete analysis of the specific clinical, histologic, immunophenotypic, molecular, treatment, and follow-up information of a large cohort of patients with anaplastic carcinoma of the pancreas is absent from the literature. Therefore, we undertook a study of 35 anaplastic carcinomas to catalogue the various characteristics in a single comprehensive study. It is the intention of this study to determine the clinical characteristics, immunophenotypic expression, cell cycle analysis, *K-ras* oncogene mutation pattern, and the treatment and clinical outcome for patients with

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Table 1. Immunohistochemistry Panel

Antigen	Primary Antibody	Manufacturer	Dilution	Antigen Recovery
Cytokeratin				
AE1/AE	mm	Boehringer Mannheim Biochemicals, Indianapolis, IN	1:50	Enzyme pretreatment
CK1		Dako, Carpinteria, CA	1:200	Enzyme pretreatment
Cytokeratin 7	mm	Dako	1:200	Enzyme pretreatment
Cytokeratin 20	mm		1:50	Enzyme pretreatment
CAM 5.2	mm	Becton Dickinson, San Jose, CA	1:50	Enzyme pretreatment
B72.3	mm	BioMed Tech, Stoughton, MA	1:20	None
CA19.9	mm	Signet Labs, Dadham, MA	1:2	
DUPAN 2	mm	BioGenex Labs, San Ramon, CA	Neat	Enzyme pretreatment
CEA	rp	Dako	1:800	
Chromogranin	mm	Boehringer Mannheim Biochemicals	1:3,200	None
Synaptophysin	rp	Dako	1:400	None
Vimentin	mm	BioGenex Labs	1:800	Microwave pretreatment
Smooth muscle actin	mm	Sigma Immuno Chemicals, St Louis, MO	1:8000	None
CD45RB	mm	Dako	1:200	Enzyme pretreatment
KP-1	mm	Dako	1:200	Enzyme pretreatment
p53	mm	Dako	1:50	Microwave recovery
Cyclin E	mm	Vector/Novocastra Labs,	1:50	Microwave recovery
p27	mm	Burlingdames, CA	1:50	Microwave recovery

Abbreviations: mm, mouse monoclonal; rp, rabbit polyclonal.

different histologic subtypes of anaplastic carcinoma of the pancreas.

Materials and Methods

Thirty-five cases of anaplastic carcinoma of the pancreas were identified in the files of the Endocrine Tumor Registry at the Armed Forces Institute of Pathology from the years 1955 to 1997. These 35 cases were identified in a review of 10,655 (0.3%) benign or malignant primary pancreatic tumors seen in consultation between 1955 and 1997. The diagnoses included in our review encompassed a variety of terms for anaplastic carcinoma, including pleomorphic carcinoma, undifferentiated carcinoma, and giant cell carcinoma. Eighteen cases were obtained from civilian sources, including university medical centers and foreign contributors, nine cases from Veterans Administration Medical Centers, and eight cases from military hospitals. Materials within the Institute's files were supplemented by a review of the patient demographics, symptoms at presentation, medical history, radiographic studies, surgical pathology reports, surgical reports, and oncology data records by specific questionnaires or direct communication with the physician or the patient.

Hematoxylin-eosin-stained slides were reviewed for all cases. All cases met the histologic criteria for anaplastic

carcinoma, as defined by the World Health Organization classification.⁸ These criteria include the four basic histologic subtypes: spindle cell, giant cell, pleomorphic giant cell, and round cell. Tumors were classified regarding the main cellular component. The number of giant cells present was semiquantitatively evaluated and separated into three groups: occasional (1/5 high power field), frequent (2-9/5 high power field), and abundant (>10/5 high power field).

Formalin-fixed, paraffin-embedded sections were stained with periodic acid-Schiff (with and without diastase digestion) and Mayer's mucicarmine. Immunophenotypic analysis was performed in 30 cases with suitable material according to the standardized avidin-biotin method of Hsu et al.⁹ Not all studies were performed on each case and, when limited, the epithelial markers were applied preferentially to document epithelial origin of the tumor cells. The broad, commercially available antibody panel is given in Table 1. Predigestion was performed for 3 minutes with 0.05% proteinase (Protease VIII; Sigma Chemical Co, St Louis, MO) in a 0.1 mol/L phosphate buffer at a pH of 7.8 at 37°C. Antigen enhancement (recovery) was performed by using formalin-fixed, paraffin-embedded tissue treated with a buffered citric acid solution and then heated for 20 minutes in a calibrated microwave oven. Following this, the sections

were allowed to cool at room temperature in a citric acid buffer solution for 45 minutes before continuing the procedure.¹⁰ Appropriate positive and negative controls were used throughout.

A positive immunoreaction was determined by chromogen deposition within the cytoplasm of the tumor cells, whereas a positive reaction with the cell cycle markers was determined by the depositing of chromogen within the nucleus. Antibody reactions were graded as weak (1+), moderate (2+), or strong (3+) staining, and the fraction of positive cells was determined by separating the percentage of positive cells into four groups: less than 10%, 10% to 50%, 51% to 90%, and greater than 90%.

Point mutations in *K-ras-2* were sought in 12 cases with sufficient and suitable material on which to perform the study. Mutational analysis was performed by topographic microdissection to yield 800 to 1,200 cells per analyte with polymerase chain reaction amplification for the *K-ras-2* exon 1 gene, flanking intron primers were used as previously described.¹¹⁻¹³ Cycle sequencing with ³⁵S was performed by use of di-deoxy terminators and one of the amplifying primers and subsequently run on a 6% denaturing polyacrylamide gel. Suspect mutations were subsequently reamplified and sequenced by use of a different primer.

Categorical variables were analyzed using chi-square tests to compare observed and expected frequency distributions. Fisher's Exact Test was used as a substitute for the chi-square test when numbers were fewer than five patients. Comparison of means between groups were made with unpaired t-tests or one-way analysis of variance, depending on whether there were two groups or more than two groups, respectively. Multiple comparisons were analyzed using the Tukey method. Confidence intervals of 95% were generated for all positive findings. The alpha level was set at $P < .05$. All analysis was conducted using Statistical Package for the Social Sciences 8.0 for PC (Chicago, IL).

Results

Clinical

The patients included 10 women and 25 men, with a male:female ratio of 2.5:1 (Table 2). Ages ranged from 34 to 85 years, with an average age at presentation of 62.5 years (median, 62 years). Twenty-six patients were white, three were black, one was Asian, and the race was unknown in five patients. Patients experienced weight loss ($n = 17$), abdominal pain, or discomfort, frequently in the right upper quadrant or epigastrium ($n = 18$), loss of appetite ($n = 5$), nausea and/or vomiting ($n = 4$); fatigue ($n = 2$); and diarrhea ($n = 2$). Four

Table 2. Patient Demographics and Clinical Presentation

Patient Characteristics	Anaplastic Carcinoma (35)
Age	
Range	34-85 yrs
Mean	62.5 yrs
Median	62.0 yrs
Gender	
Women	10
Men	25
Race	
White	26
Black	3
Asian/Indian	1
Unknown	5
Symptoms	
Weight loss	17
Abdominal pain	18
Nausea/vomiting	4
Loss of appetite	5
Fatigue	2
Diarrhea	2
Palpable mass	4
Jaundice	4
Diabetes	1
Duration of symptoms (range)	3 d to 52 wks
Duration of symptoms (mean)	13.2 wks

patients each presented with a palpable mass and with jaundice. The duration of symptoms ranged from 3 days to 1 year, with an average of 13.2 weeks. The clinical presentation did not have a bearing on the outcome of the patient ($P = .387$). On average, there was a much shorter duration of symptoms for patients with tumors with a dominant spindle cell component (6 weeks) when compared with the average for patients with tumors with pleomorphic large cells (14.1 weeks), but this difference is not statistically significant.

Radiographic Investigation

Roentgenographic procedures were performed in the majority of patients, with computer tomography and ultrasound the most frequently used. Cross-sectional images identified a diffuse enlargement of the pancreas, or a mass involving the pancreas. Erosion of the organ margins or obliteration of the fat planes suggested a malignant diagnosis. Degenerative changes could be identified in many of the tumors.

Table 3. Location and Size of Tumors

Tumor Characteristics	Anaplastic Carcinoma
Location	
Head of pancreas	17
Tail of pancreas	10
Body of pancreas	3
Body and tail of pancreas	2
Entire pancreas	3
Size (cm)	
Range	2.5–20.0
Average	9.2

Pathology

Macroscopic. The majority of tumors ($n = 17$) were located in the head of the pancreas (Table 3). An additional three occurred in the body, 10 in the tail, two in both the body and tail, and three involved the entire pancreas. Tumor size ranged from 2.5 to 20 cm, with an average size of 9.2 cm. While the pleomorphic subtype tended to be larger on average (pleomorphic, 9.8 cm; spindle cell, 4.9 cm), there was no statistically significant difference between these subtypes of anaplastic carcinoma based on size, nor was there a worse clinical outcome ($P = .170$). The tumors were firm to rubbery ($n = 21$), soft to fleshy ($n = 5$), or cystic ($n = 8$). The cystic cavities were filled with opaque, brown, hemorrhagic, thick material. Ten tumors yielded macroscopically visible necrotic debris, while an additional 10 cases showed old blood when the tumors were serially sectioned. The surrounding pancreatic tissue was generally atrophic.

Microscopic. The tumors were composed of an assortment of growth patterns and cell types interspersed between one another. Even though there was a remarkable heterogeneity within each specific subtype of anaplastic carcinoma, in aggregate a single cell type predominated (each of these subtypes will be described in detail below). All the tumors were widely invasive, infiltrating into the surrounding pancreatic parenchyma and extrapancreatic soft tissues, as well as being identified around nerves (perineural invasion) and within vascular and lymphatic channels. Direct extension of the tumor into surrounding organs was documented in 16 tumors (duodenum, 7; adrenal, 2; spleen, 4; hepatic porta, stomach, and kidney; 1 each). Furthermore, 20 cases showed metastatic disease (systemic metastases, 15; lymph nodes, 14).

The liver ($n = 13$) and lungs ($n = 10$) were affected by metastatic disease most frequently, followed by peritoneum ($n = 7$), adrenals ($n = 5$), and heart, spleen, brain, vertebral, pleural, thyroid (1 each).

Large-Cell Type ($n = 31$). The cells were arranged in nests, made up of discohesive, large, pleomorphic cells without any significant background stroma (Fig 1, top). The tumor cells were large, round to polygonal, frequently irregular in shape, with abundant eosinophilic cytoplasm (Fig 1A). Focal spindling (10% to 30%) of the tumor cells was observed in 11 tumors. Rare, isolated, osteoclastic-type giant cells were noted in five tumors. The majority of cases ($n = 22$) contained multinucleated giant cells, either of foreign body type (peripheral nuclei) or tumor giant cells (centrally placed atypical nuclei). Furthermore, phagocytic activity was common ($n = 22$) (Fig 1, bottom). Malignant giant cells had engulfed other tumor cells (cannibalism) and/or inflammatory cells (Fig 1B), while the foreign body giant cells tended to engulf tumor cell or inflammatory cell breakdown products. Two cases showed areas of squamous differentiation. The origin of these anaplastic cells (pleomorphic cells) from an epithelial primordia was partially supported by the presence of infiltrating ductal adenocarcinoma at the periphery of the tumor mass ($n = 12$). The production of mucin by the anaplastic tumor cells was confirmed with a positive mucicarmine reaction in 10 of 31 cases tested. Chronic pancreatitis could be identified in the uninvolved surrounding pancreas in nine cases.

Spindle-Cell Type ($n = 4$). These tumors were composed largely of spindle cells arranged in a loose storiform pattern. These tumor cells were more monotonous and uniform than those of the large-cell type, but still had a degree of variation in shape and size (Fig 2). Tumor giant cells and squamous metaplasia were identified in one tumor each. One of the cases contained an infiltrating ductal adenocarcinoma (documented by the presence of gland profiles and mucin production).

Immunohistochemistry

Eighteen of the tumors tested ($n = 23$) were reactive with at least one epithelial marker (Table 4; Fig 3, top), coexpressing vimentin in 15 cases (Fig 3, bottom). Dupan 2, CA19-9, and carcinoem-

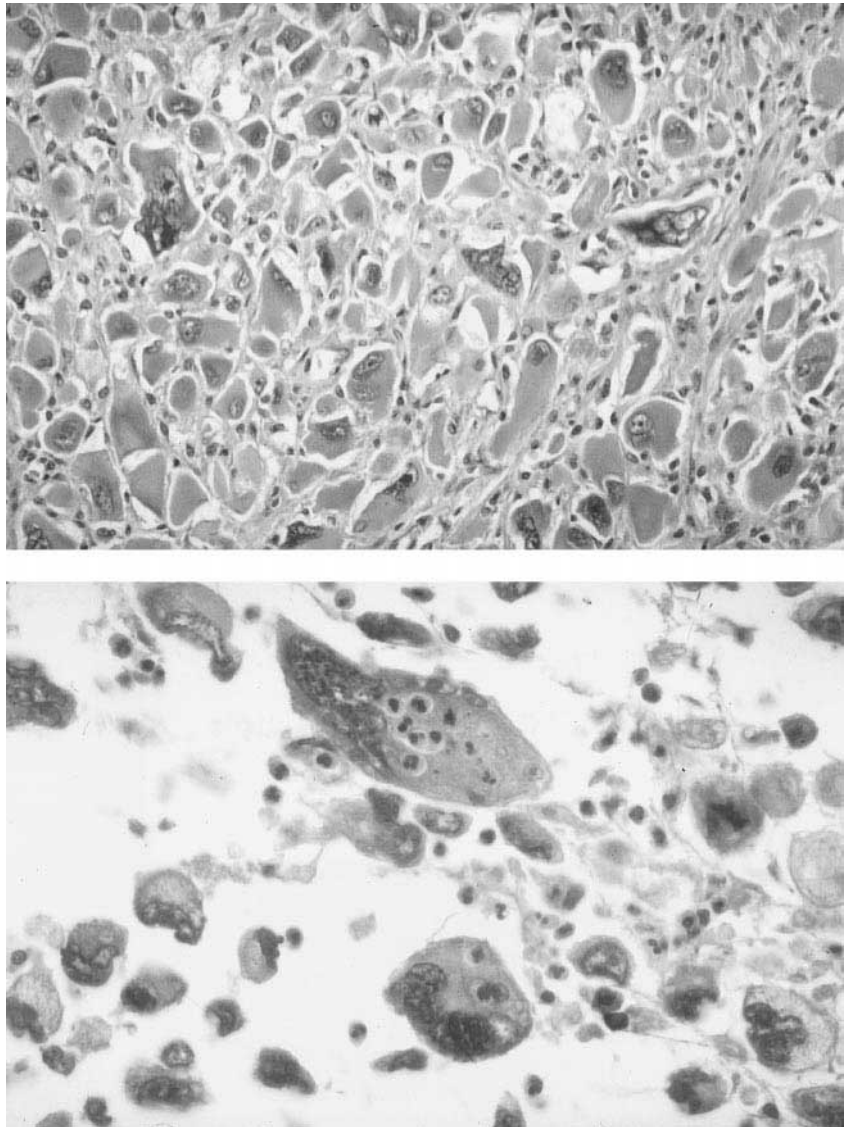


Figure 1. (Top) Large cell type of anaplastic carcinoma of pancreas showing remarkable nuclear atypia. (Bottom) Discohesive large tumor cells with phagocytosis of inflammatory cells.

bryonic antigen were variably reactive. CD68 (KP-1) was reactive in tumor giant cells in two cases. p53 was moderately to strongly reactive in a limited number of cells in the majority of cases tested ($n = 10/14$). Reactivity with p27 equaled or exceeded the reactivity of cyclin E in all positive cases, except for one case (nonreactive with p27; reactive with cyclin E). There was no reactivity with CK20, B72.3, chromogranin, synaptophysin, or smooth muscle actin. Because a limited number of slides were available, keratin and vimentin were the only immunohistochemical studies performed in the three patients who were alive without evidence of disease.

Furthermore, the three patients who had died without evidence of disease also had immunoreactivity with keratin, DUPAN2, and vimentin, but other markers were nonreactive or there was insufficient material on which to perform the tests.

***K-ras-2* Oncogene Determination**

Eight of the 12 tumors tested for *K-ras-2* oncogene showed a mutation, while the remaining four patients did not show a mutation and were of wild type. All the mutations were glycine to valine on codon 12, chromosome 12p. The eight mutated

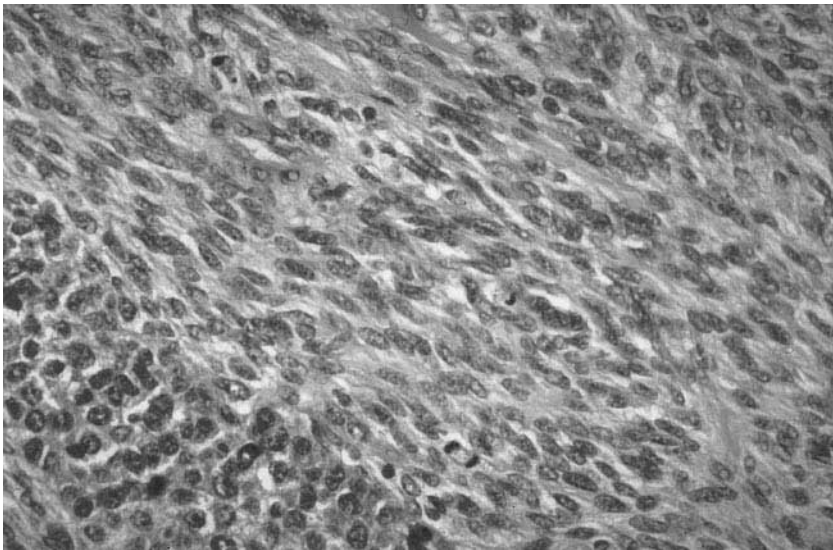


Figure 2. Spindled cells are arranged in a fascicular growth pattern. Mitotic figures are noted in this spindle cell type of anaplastic carcinoma.

cases occurred in patients with both large cell type ($n = 7$) and spindle cell type ($n = 1$), all of whom died with an average survival of 1.9 and 1 month, respectively. Mean overall survival for the patients with *K-ras* mutations was 1.7 months. All of the patients with wild type ($n = 4$) had large cell histomorphology and died with disease after an average survival of 9.5 months. The difference in survival between patients with and without *K-ras* oncogene mutations was not statistically significant ($P = .173$).

Clinical Outcome

Follow-up information was available in all 35 patients (Table 5). Three patients were alive at last follow-up, two without evidence of disease (14.6 and 7.2 years, respectively), and one with recurrent tumor (follow-up 14.7 years), all with the large cell type. Three patients, all with large cell type, had died without evidence of disease of unrelated causes after an average survival of 4.7 years.

The remaining 29 patients died with disseminated tumor (average, 5.5 months). Patients with the large cell type had a slightly longer survival (6.0 months) when compared with patients with the spindle cell type (3.3 months). While this difference was statistically significant, the difference is not clinically significant. As previously stated, there was no statistically significant difference in survival based on *K-ras* oncogene mutation.

There was no statistically significant difference in

outcome between sites of metastatic disease ($P = .778$), tumor type ($P = .559$), squamous metaplasia ($n = 3$; $P = .690$), infiltrating adenocarcinoma ($n = 13$; $P = .634$), or chronic pancreatitis ($n = 11$; $P = .480$). Patients who had tumors with abundant giant cells tended to have a longer survival (16.4 months) than patients with frequent (8.0 months) or occasional giant cells (5.4 months). While these differences are statistically significant ($P = .011$), it is probably not clinically significant with such a short overall survival.

Discussion

Anaplastic carcinomas of the pancreas are infrequent tumors, accounting for less than 10% of all pancreatic tumors.¹⁻⁶ A number of terms have been applied to these tumors, including pleomorphic carcinoma, pleomorphic giant cell carcinoma, pleomorphic large cell carcinoma, sarcomatoid carcinoma, and small cell carcinomas^{14,15} Anaplastic foci are identified in ductal adenocarcinomas⁵ and in ectopic pancreata, but are not the dominant pattern of growth.¹⁶

Although a variety of patterns can be present within each tumor, Alguacil-Garcia and Weiland⁷ divided anaplastic carcinomas into four groups: (1) spindle cell (spindle cells dominate with occasional giant and bizarre cells); (2) osteoclastic giant cell; (3) pleomorphic giant cell (mono- and multinucleated pleomorphic giant cells); and (4) round cell anaplastic (small, round, and uniform cells, inter-

Table 4. Immunohistochemical Reactions

Antibody	No. of Cases With Positive Reactions*	No. of Cases Positive by Percentage of Cells	No. of Cases Positive by Intensity of Reaction
Keratin	14/22	9, >90%	2 = 1+
		3, 51%-90%	1 = 2+
		1, 10%-50%	11 = 3+
		1, <10%	
CK 7	9/16	5, >90%	1 = 1+
		3, 51%-90%	8 = 3+
		1, <10%	
CK 20	0/20	N/A	N/A
CAM 5.2	8/16	1, 51%-90%	4 = 1+
		5, 10%-50%	3 = 2+
		2, <10%	1 = 3+
B 72.3	0/16	N/A	N/A
CA 19-9	5/18	1, 51%-90%	2 = 1+
		2, 10%-50%	3 = 3+
		2, <10%	
DUPAN 2	7/18	3, 10%-50%	1 = 1+
		4, <10%	1 = 2+
			5 = 3+
CEA	8/21	8, <10%	1 = 1+
			2 = 2+
			5 = 3+
Chromogranin	0/17	N/A	N/A
Synaptophysin	0/13	N/A	N/A
Vimentin†	18/21	2, >90%	2 = 1+
		4, 51%-90%	3 = 2+
		5, 10%-50%	13 = 3+
		7, <10%	
SMA	0/13	N/A	N/A
KP-1	5/15	4, >90%	1 = 1+
		1, <10%	1 = 2+
			3 = 3+
CD45RB	0/15	N/A	N/A
p53	10/14	2, >90%	2 = 1+
		2, 51%-90%	1 = 2+
		2, 10%-50%	7 = 3+
		4, <10%	
Cyclin E‡	2/13	1, 10%-50%	2 = 1+
		1, <10%	
p27‡	5/13	3, 10%-50%	3 = 1+
		2, <10%	2 = 2+

Abbreviation: N/A, not applicable.

* Thirty cases had immunophenotypic analysis performed, although not all antibodies were used in each case, with only a single antibody tested in a few cases because of limited archival material.

† Fifteen vimentin-positive tumors coexpressed keratin (n = 12), CK7 (n = 7), or CAM 5.2 (n = 6).

‡ Reactivity with p27 equaled or exceeded the reactivity with cyclin E in all positive cases.

mingled with occasional eosinophilic plump cells and giant cells). Osteoclastic giant-cell type is classified by the World Health Organization as a separate entity and were not considered in this discussion. We believe that small, round-cell tumors^{14,17,18} of the pancreas are secondary lesions with multifocal tumor masses, usually metastatic small cell carcinomas of the lung, malignant lymphomas, or primary endocrine tumors. This assertion is further supported by the literature, in which pulmonary primaries are not excluded^{19,20} or islet cell tumors are confirmed.²¹

Anaplastic carcinomas do not preferentially involve a specific part of the pancreas, even though we had more cases present in the head of the pancreas, this difference was not statistically significant (Table 3). These findings are different from the literature,^{5-7,22} although in agreement with Guillan and McMahan.²³ Cyst formation occurred within nine of the tumors in our series, a finding that has been previously described.^{24,25} Furthermore, cystic tumors (mucinous cystic neoplasm) can have associated anaplastic carcinoma.²⁶⁻³⁰ All tumors show hemorrhage and/or necrosis.¹⁵

Fundamental to the diagnosis of anaplastic carcinoma are the large, bizarre, frequently multinucleated malignant cells, containing abundant, eosinophilic cytoplasm and lacking a supporting stroma.^{1,15,31} The "giant cells" are malignant tumor cells, although osteoclast-type giant cells also can be present, as our study suggests.²⁵ The presence alone of osteoclast-type giant cells does not represent a malignant finding, as they may occur in a variety of benign conditions (eg, abscesses, hemorrhage, necrosis, pseudocysts, fungal infections, and sarcoidosis).^{32,33} The various types of giant cells may be related,³⁴ arise from a multipotential stem cell,³² or represent a "sarcomatous metaplasia" of the carcinoma cells.^{5,35-43} The frequent expression in our series of malignant giant cells for CK7 seems to support an epithelial origin^{3,7,36-39,44} or dedifferentiation of a ductal adenocarcinoma, rather than an acinar differentiation,⁴⁵ especially in light of the associated ductal adenocarcinoma.^{5,7,8,22,36,37,39-41,46} Phagocytosis of erythrocytes or tumor cells (cannibalism) is a common feature in all anaplastic carcinomas.^{2,5,7,22,31,37,47,48}

The tumor giant cells must be distinguished from reactive histiocytes and foreign body giant cells, as well as the cells of metastatic carcinoma, malignant melanoma, choriocarcinoma, Hodgkin's

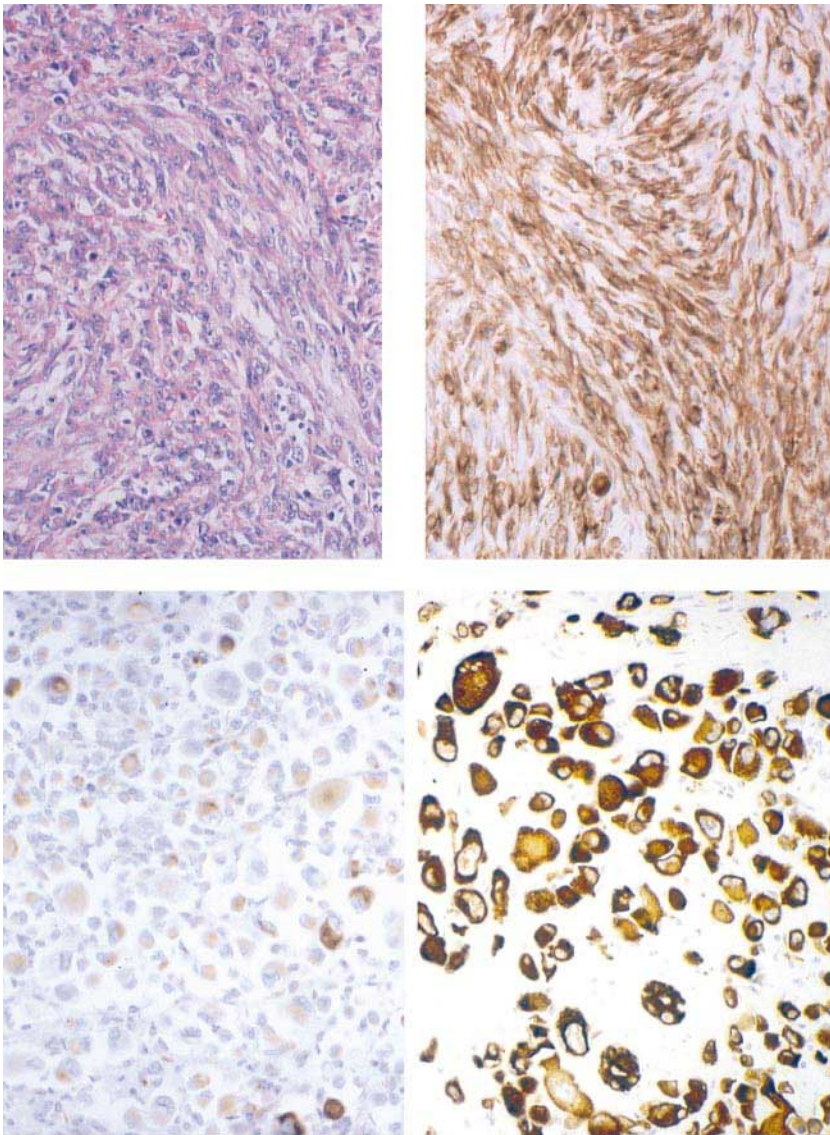


Figure 3. (Top) There was strong immunoreactivity for keratin (right) in this spindle cell type of anaplastic carcinoma (left). (Bottom) The tumor cells of the large cell type of anaplastic carcinoma were immunoreactive with antibodies for vimentin (left) and keratin, specifically CK7 (right).

lymphoma, anaplastic large cell lymphoma, epithelioid sarcomas, and malignant fibrous histiocytoma.^{6,7,33,49} An immunohistochemical evaluation can assist in distinguishing cells of anaplastic carcinoma of the pancreas from the tumors listed above.

In our series, tumor cells were usually reactive with at least one epithelial marker (EMA, CK7, cytokeratin cocktail; n = 18/23), supporting the studies in

Table 5. Patient Outcome and Average Survival for Anaplastic Carcinoma

Average Survival	Large Cell (31 Patients)	Spindle Cell (4 Patients)	K- <i>ras</i> Mutation (8 Tumors)
Alive, no evidence of disease	2 (6.5%), 133.3 mo	0	0
Alive, recurrent disease	1 (3.2%), 179.4 mo	0	0
Dead, no evidence of disease	3 (9.7%), 57.7 mo	0	0
Dead, with disease	25 (80.6%), 6.0 mo	4 (100%), 3.3 mo	8 (100%), 1.7 mo

the literature,^{22,24} while also coexpressing vimentin.^{28,30,34,35,37,39-42,50-56} The presence of infiltrating ductal adenocarcinoma in the cases that did not have blocks on which to perform the immunohistochemical analyses was deemed as support of an epithelial origin for the anaplastic carcinoma, although we were not able to completely exclude a malignant lymphoma (anaplastic large cell type) or a malignant melanoma.

Our detected *K-ras* mutation rates (66.7%) were in the range of those reported in the literature for anaplastic carcinoma.^{32,38,39,47,53,57} *K-ras* mutations are identified in a high percentage of pancreatic cancers (reported up to 100%⁵⁸), usually increasing in frequency with greater cytologic atypia, which is suggested to affect early pancreatic carcinogenesis, resulting in a dismal prognosis.^{11-13,59-64} The findings in our series support a poor prognosis and a relatively shorter survival (1.7 months) in patients with a *K-ras* mutation than those without (9.5 months). However, this finding is not statistically significant, and this small difference of a few months is not clinically germane.

We tried to evaluate the prognostic role of the cell cycle regulatory proteins, cyclin E and p27. While tumor development and growth regulation is not well understood, cyclin E and p27 play an important role in cell cycle regulation. Cyclins (proteins) regulate the activity of cyclin-dependent kinases (CDK), which are active only when bound to cyclins.^{65,66} Cyclin-CDK complexes propel the cell into S phase or mitosis, depending on their specificity. The major cyclin groups (G1 cyclins A, D, and E, and G2 cyclins A and B) affect proliferation depending on the phase of the cell cycle affected. G1 cyclins bind to CDK in the G1 phase and are required for entering the S phase. G2 cyclins are necessary for entering the M phase.^{65,66} Cyclin E acts at the G1-S transition, with p27 counteracting the effects of cyclin E, because it is a stoichiometric inhibitor of the cyclin-CDK complex (along with p15) in the G1 phase.^{67,68}

High cyclin E and low p27 levels are associated with a significant increase in mortality in breast cancer patients, although each marker is considered an independent prognostic marker.⁶⁹⁻⁷¹ Similarly, a lack of p27 expression is associated with a worse prognosis in colorectal and gastric carcinomas.^{72,73} Unfortunately, all our cases showed a weak reactivity with both antibodies (except for

one case), which did not yield a specific relationship to histologic subtype or clinical outcome.

Similar to the *K-ras* mutation results, there is variable detection of p53 protein mutations in anaplastic carcinomas.^{57,74,75} It has been proposed that *K-ras* mutations may exceed the frequency of p53 mutations in pancreatic cancer in general.^{57,74,76} However, in contrast to these findings, we identified the presence of presumed p53 mutations in 10 of 14 cases tested, with less than 10% to more than 90% of the tumor cells showing the abnormal phenotype by immunohistochemistry, with most showing 2+ to 3+ reactivity (Table 4). This finding may support the assertion in the literature that suggests a worse prognosis associated with p53 protein abnormalities.^{58,75-79}

The patients in our series, in order of frequency, showed metastatic disease to liver, lung, and peritoneum, irrespective of the anatomic region of the pancreas affected by the primary tumor. Our results are different from the literature, which states that body and tail tumors are more likely to give pulmonary rather than hepatic metastases,⁴¹ and that the incidence of pulmonary metastases is higher in anaplastic carcinoma than in conventional ductal adenocarcinoma.^{5,6,40} In our cases, direct spread to the surrounding soft tissues, stomach, spleen, and peritoneum was similar to those reported in the literature.^{6,80} While infrequent, metastatic deposits were occasionally better differentiated than the primary tumor.

The prognosis for anaplastic carcinoma, both subtypes considered, is worse than the average survival of patients with invasive ductal adenocarcinoma, not further specified of 10 to 20 months, with less than 3% 3-year survival.^{22,40,41,81} Although the number of months is not significantly different statistically, nor perhaps clinically important, individual tumor subtypes may have a limited difference in patient survival. With so few cases and a limitation in statistical analysis, we cannot be certain that the pleomorphic large cell type has a better prognosis than the spindle cell type, even though we have more cases than reported in the literature.^{15,33,43,51} Overall tumor size is not a reliable prognostic indicator.⁸²

In summary, anaplastic carcinomas of the pancreas generally occur with greater frequency in the head of the pancreas, usually in older men. The epithelial nature and ductal origin of the tumors can usually be confirmed by immunohistochemical

studies and the frequent and strong association with infiltrating ductal adenocarcinoma. Prognostic markers and *K-ras* oncogene mutations support a proliferative tumor that has a poor prognosis irrespective of the various subtypes of large cell, osteoclastic giant cell, or spindle cell type. Irrespective of the treatment, the overall prognosis for this tumor remains grave.

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