The American Journal of Surgical Pathology *26(5): 551–566, 2002*

Pheochromocytoma of the Adrenal Gland Scaled Score (PASS) to Separate Benign From Malignant Neoplasms

A Clinicopathologic and Immunophenotypic Study of 100 Cases

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No comprehensive series has evaluated the histologic features of pheochromocytoma to separate benign from malignant pheochromocytoma by histomorphologic parameters only. Fifty histologically malignant and 50 histologically benign pheochromocytomas of the adrenal gland were retrieved from the files of the Armed Forces Institute of Pathology. The patients included 43 females and 57 males, with an age range of 3–81 years (mean 46.7 years). Patients usually experienced hypertension (n = 79 patients). The mean tumor size was 7.2 cm (weight was 222 g). Histologically, the cases of malignant pheochromocytomas of the adrenal gland more frequently demonstrated invasion (vascular [score = 1], capsular [score = 1], periadrenal adipose tissue [score = 2]), large nests or diffuse growth (score = 2), focal or confluent necrosis (score = 2), high cellularity (score = 2), tumor cell spindling (score = 2), cellular monotony (score = 2), increased mitotic figures (>3/10 high power fields; score = 2), atypical mitotic figures (score = 2), profound nuclear pleomorphism (score = 1), and hyperchromasia (score = 1) than the benign tumors. A Pheochromocytoma of the Adrenal gland Scaled Score (PASS) weighted for these specific histologic features can be used to separate tumors with a potential for a biologically aggressive behavior (PASS ≥4) from tumors that behave in a benign fashion (PASS <4). The pathologic features that are incorporated into the PASS correctly identified tumors with a more aggressive biologic behavior. Application of these criteria to a large cohort of cases will help to elucidate the accuracy of this grading system in clinical practice.

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Presented at the 90th Annual Meeting of the United States and Canadian Academy of Pathology, Atlanta, Georgia, March 5–10, 2001.

The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of Defense.

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Key Words: Adrenal—Pheochromocytoma—Malignant—Benign—Grading—Prognosis.

Am J Surg Pathol 26(5): 551–566, 2002.

The term pheochromocytoma, a catecholamine-secreting tumor arising from the chromaffin cells of the sympathoadrenal system, was coined by Poll in 1905 to describe the dusky (pheo) color (chromo) of the cut surface of the tumor when exposed to dichromate.[23] The vast majority of pheochromocytomas arise from the adrenal medulla where the largest collection of chromaffin cells are found,[27] whereas the term paraganglioma is used for this same tumor in other anatomic sites (except for the organs of Zuckerkandl where it is referred to as extraadrenal pheochromocytoma). It is commonly accepted that the biologic behavior of pheochromocytomas cannot be predicted on the basis of macroscopic or microscopic features alone. Indeed, without documented metastatic disease (defined by the presence of tumor deposits in sites that normally do not contain chromaffin cells to exclude multicentric disease), the prospective diagnosis of a malignant pheochromocytoma has been considered nearly impossible.[2,8,9,14,17,31–33,36–38,50] A few authors[12,14,18,19,28,33,35,45] have suggested that necrosis, vascular invasion, extensive local invasion, and mitotic figures may indicate a malignant pheochromocytoma. Case reports and small series individually serve as a valuable adjunct to understanding malignant pheochromocytomas of the adrenal gland (MPA). Experience with diagnosis and treatment of MPA remain limited because a comprehensive analysis encompassing the use of clinical, radiographic, histologic, immunochemistry, and follow-up information applied to a group of MPA is, to the best of my knowledge, absent from the literature

(MEDLINE 1966–2001). Therefore, it is the intention of this study to provide a comprehensive analysis of pheochromocytomas encompassing the use of clinical features, morphologic findings, immunophenotypic studies, and patient follow-up information (including staging and adjuvant therapies) applied to a group of 100 patients with this tumor to better identify the parameters that may assist in distinguishing benign from MPA and thereby help to predict prognosis by applying the Pheochromocytoma of the Adrenal Gland Scaled Score (PASS).

MATERIALS AND METHODS

The records of 777 patients with tumors diagnosed as "pheochromocytoma," "malignant pheochromocytoma," "pheochromocytoma with malignant features," "pheochromocytoma with atypical features," and "atypical pheochromocytoma" were identified in the files of the Endocrine Registry at the Armed Forces Institute of Pathology from 1970 to 1997. These 777 patients were identified in a review of 7983 patients with benign adrenal neoplasms and primary adrenal or metastatic malignant neoplasms to the adrenal gland seen in consultation during this same time period. Fifty patients had tumors diagnosed as MPA, representing 1.2% of the 4027 benign or malignant primary adrenal gland neoplasms (no metastatic tumors included) diagnosed during the above reference period. The classification of "malignant" was based on the histologic criteria and not on the clinical behavior alone because 17 patients had a benign clinical course and 33 developed pathologically documented metastatic disease. As a point of comparison, 727 benign pheochromocytomas were diagnosed during the same time period. Only the first 50 patients in whom complete follow-up of at least 10 years could be obtained were histologically examined. Therefore, a total of 100 patients with pheochromocytomas (benign or malignant) compose the subject of this study based on complete and adequate follow-up information and hematoxylin and eosin-stained slides to make a definitive diagnosis. Sixty-five cases were obtained from civilian sources, including university medical centers and foreign contributors, 25 cases from military hospitals, and 10 cases from Veterans Administration medical centers.

Materials within the Institute's files were supplemented by a review of the patient demographics (gender, age); symptoms and physical findings at presentation (diaphoresis, headaches, palpitations, weakness, syncope or dizziness, anxiety, flushing, chest pain, nausea, hypertension including paroxysmal type, vomiting, and weight loss), including duration; and medical and surgical history (specifically related to a family history of similar tumor development, or the presence of von Hippel-Lindau disease, von Recklinghausen's disease, neurofibromatosis, or multiple endocrine neoplasia syndrome [MEN], i.e., nonsporadic cases). Laboratory values, specifically related to catecholamines (serum or urine) and their various metabolites, were obtained when available. In addition, we reviewed radiographic, surgical pathology, and operative reports and obtained follow-up information from oncology data services by written questionnaires or direct communication with the treating physician(s) or the patient. Follow-up data, available for all patients, included information regarding tumor location, presence of metastatic disease (local lymph nodes, distant lymph nodes, or other organ sites), treatment modalities used, and the current patient and disease status. This clinical investigation was conducted in accordance and compliance with all statutes, directives, and guidelines of the Code of Federal Regulations, Title 45, Part 46, and the Department of Defense Directive 3216.2 relating to human subjects in research.

Hematoxylin and eosin-stained slides from all patients were reviewed for morphologic assessment of MPA and benign pheochromocytoma of the adrenal gland (BPA). A number of macroscopic and histologic observations were recorded for each tumor as follows: tumor weight (in grams); tumor size (greatest dimension in centimeters) (Fig. 1); tumor encapsulation (presence or absence); capsular invasion (Fig. 2); vascular invasion (Fig. 2), defined by direct extension into the vessel lumen, intravascular attached tumor thrombi, and/or tumor nests covered by endothelium identified in a capsular or extracapsular vessel (i.e., tumor plugs in vascular spaces within the tumor mass did not qualify as vascular invasion) [no distinction was made between veins or lymphatic channels for purposes of this study, and we did not require that the exact point of penetration into a vessel be recognized to qualify as evidence of vascular invasion]; extension into the periadrenal adipose tissue; cell nests (small, "zellballen-type" nests [Fig. 3] to large, confluent nests [a "large nest" was defined as three to four times the size of a "zellballen" or the normal size of the medullary paraganglia nests; (Fig. 4) or diffuse growth; necrosis (individual cell, focal [identified in the center of large nests] (Fig. 4), or confluent and diffuse type, with percent necrosis of the tumor area); fibrosis; degenerative changes; calcifications; cellularity (subjectively divided into low [greater amount of area accounted for by a few cells with ample cytoplasm], moderate [intermediate between low and high], and high [greater amount of area accounted for by many cells with a high nuclear-to-cytoplasmic ratio); cytoplasmic quality (clear, basophilic, eosinophilic, amphophilic); tumor spindling (when present, the percent of tumor area spindled) (Fig. 5 and Fig. 6, right); cellular pleomorphism (mild, moderate, profound) (Fig. 6, upper left); cellular monotony; nuclear hyperchromasia; pyknosis; nucleoli (defined as >4 μm in diameter, eosinophilic-magenta, or irregular in shape) (Fig. 6, upper left); intranuclear cytoplasmic in-

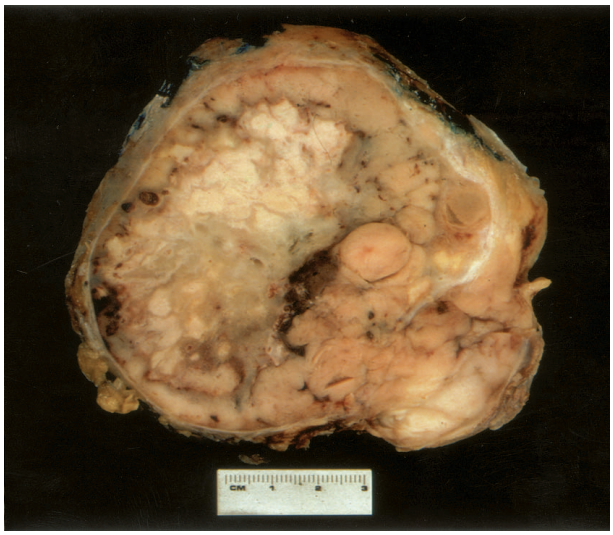

FIG. 1. Macroscopic view of a malignant pheochromocytoma, demonstrating capsular invasion, hemorrhage, necrosis, and multinodularity.

clusions; intracytoplasmic eosinophilic hyaline globules; giant cells (present and type of giant cells; multinucleated, foreign body-type, osteoclast-type, or peculiar neoplastic cells) (Fig. 6, upper left); mitotic figures (number of mitoses per 10 high power fields [HPF] [magnification at ×40 with a ×10 objective lens using an Olympus BX40 microscope]); atypical mitotic figures (present or absent, and defined by abnormal chromosome spread, tripolar or quadripolar forms, circular forms, or indescribably bizarre) (Fig. 6, lower left); brown fat (identified in the periadrenal adipose tissue); amyloid (acellular, amorphous, eosinophilic extracellular matrix material); and if metastatic disease were present, whether it was histologically similar to the primary tumor.

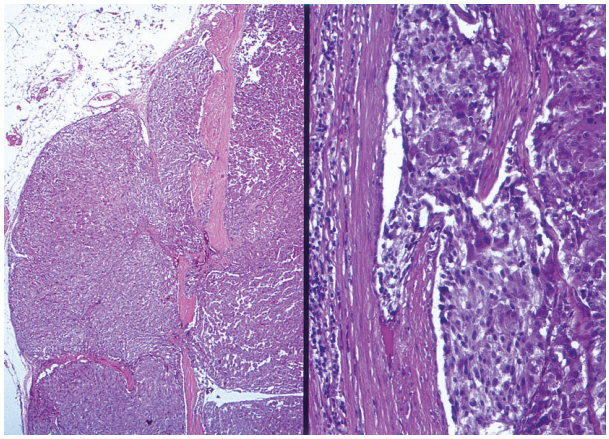

FIG. 2. A thick fibrous capsule is transgressed by the neoplastic cells with extension into the surrounding adipose connective tissue (left) in this malignant pheochromocytoma. Extension into a vascular spaces is noted in a malignant pheochromocytoma (right).

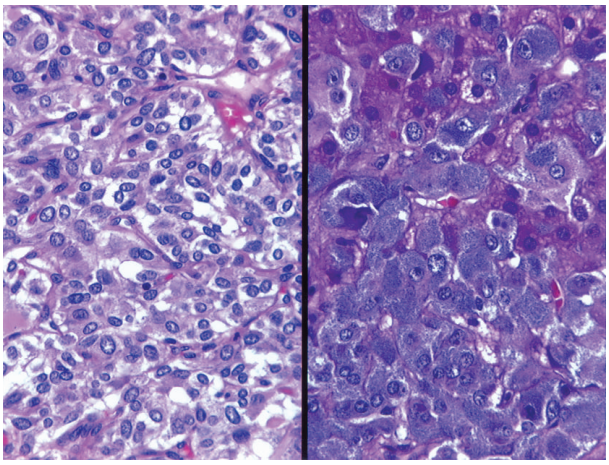

FIG. 3. The characteristic small nests ("zellballen") pattern was seen in all pheochromocytomas, whether benign or malignant. A granular, basophilic cytoplasm was usually identified surrounding slightly irregular nuclei (right).

A PASS was developed by taking the patients whose tumors were clinically and histologically malignant and identifying the histologic features that were uniquely present or present in a greater frequency than in the patients whose tumors were clinically and histologically benign. Features that were present in both benign and malignant tumors were given a lower weight, whereas those features identified only in malignant neoplasms or to a much greater frequency in malignant neoplasms were given a heavier weight. No one histologic feature was uniquely able to identify a tumor that would behave in an aggressive clinical fashion. Therefore, a scaled score was developed to account for variability in processing, sectioning, and ease of being able to quantify or qualify morphologically reproducible criteria. A PASS of <4 accurately identified all histologically benign and clinically benign tumors (Table 1). A PASS of ≥4 correctly identified all tumors that were histologically ma-

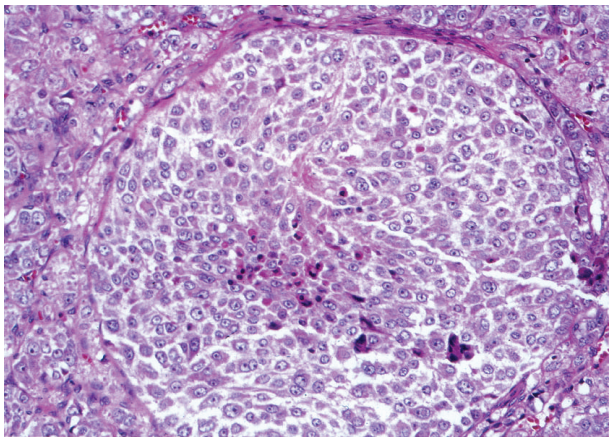

FIG. 4. A large nest is seen juxtaposed to small nests ("zellballen"). An area of central comedonecrosis is also appreciated.

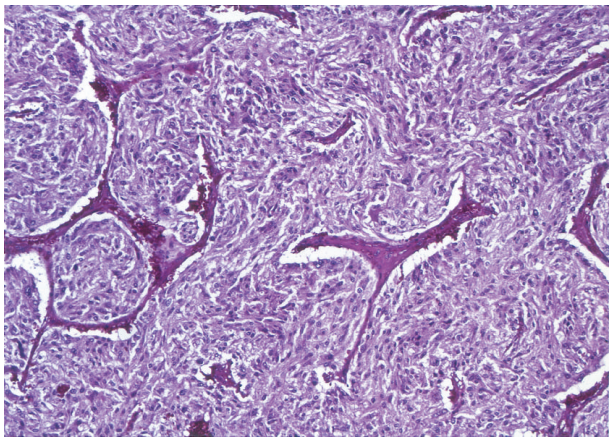

FIG. 5. Large nests showing tumor cell spindling in a malignant pheochromocytoma.

lignant, even though 17 of the 50 patients did not develop malignant clinical behavior. However, all of the patients who had clinically aggressive neoplasms were identified by a PASS of ≥4.

Immunophenotypic analysis was performed in 76 cases with suitable material by the standardized avidin-biotin method of Hsu et al.[7] using 4-μm-thick, formalin-fixed, paraffin-embedded sections. Table 2 documents the pertinent, commercially available immunohistochemical antibody panel used. The analysis was performed on a single representative block in each primary tumor and on a single block of a metastatic focus, if available. When required, proteolytic antigen retrieval was performed by predigestion for 3 minutes with 0.05% Protease VIII (Sigma Chemical Co., St. Louis, MO, USA) in a 0.1 M phosphate buffer, pH 7.8, at 37°C. Cellular conditioning to achieve antigen enhancement (recovery) was performed by using formalin-fixed, paraffin-embedded tissue treated with a buffered citric acid solution and heated for 20 minutes in a calibrated microwave oven. Afterward, the sections were allowed to cool at room temperature in a citric acid buffer solution for 45 minutes before continuing the procedure. Standard positive controls were used throughout, with serum used as the negative control. The antibody reactions were graded as weak (1+), moderate (2+), and strong (3+) staining, and the fraction of positive cells was determined by separating the percentage of positive cells into four groups: <1%, 1–10%, 11–90%, and >90%, spe-

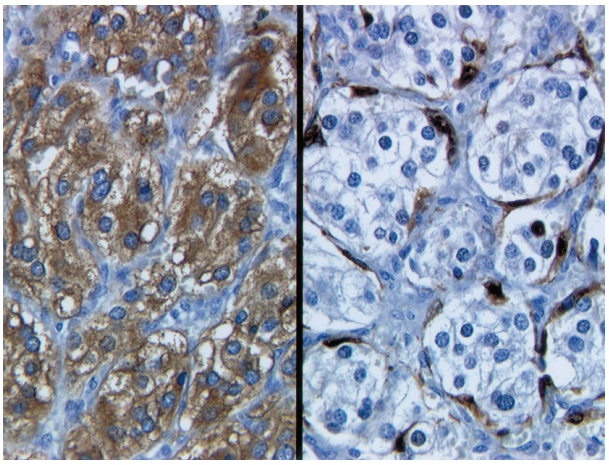

FIG. 7. The characteristic chromogranin (left) immunoreactivity in the pheochromocytes is contrasted to the S-100 protein immunoreactivity of the supporting sustentacular supporting cells (right) in this benign pheochromocytoma.

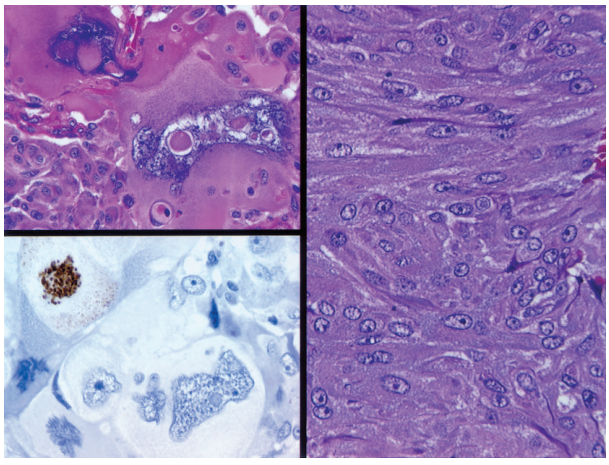

FIG. 6. The upper left image demonstrates profound nuclear pleomorphism with prominent nucleoli and intranuclear cytoplasmic inclusions. Small pheochromocytes are noted in the lower corner of this image. The lower left image demonstrates an atypical mitotic figure, highlighted with a Ki67 immunohistochemical study. The right image demonstrates tumor cell spindling associated with the basophilic granular cytoplasm of a malignant pheochromocytoma.

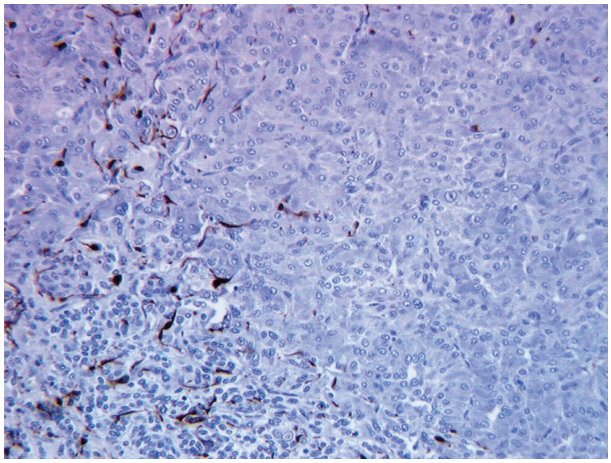

FIG. 8. An S-100 protein immunohistochemical study shows obvious immunoreactivity in the "zellballen" part of the tumor with almost complete loss in the large nest or diffuse area of a malignant pheochromocytoma.

cifically for the proliferation markers (p53 and Ki67) and S-100 protein.

The review of the literature was extensive, based on a MEDLINE search from 1966 to 2001 with a few specific earlier studies or foreign language articles included for balance and background. However, for purposes of succinctness, the review was limited to pheochromocytomas identified in surgical pathology material that included demographic, histopathologic, immunophenotypic, and/or treatment information written in English and specifically related to malignant pheochromocytoma criteria.

Categorical variables were analyzed using χ^2 tests to compare observed and expected frequency distributions. 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. Linear regression was used to investigate two measured variables, and Pearson correlation coefficients were generated to measure the strength of the association. Confidence intervals of 95% were generated for all positive findings. The alpha level was set at $p < 0.05$. All analyses were conducted using the Statistical Package for the Social Sciences software (version 8.0 for PC; Chicago, IL, USA).

RESULTS

Clinical Demographics and Presentation

Benign

A summary of the clinical information on the patients in this series is provided in Table 3. The mean age at presentation for the patients who had an associated syndrome or genetic abnormality was of a young age at 35.5 years ($p = 0.005$). Patients presented with a variety of symptoms and physical findings, but the most frequently identified physical finding was hypertension ($n = 39$ patients) (Table 3). The hypertension was described as episodic, labile, and erratic (paroxysmal), frequently associated with postural changes, as well as being sustained. Most patients presented with other associated findings as noted in Table 3. When pain was encountered it was described as dull and aching to sharp and stabbing and perceived in the abdominal cavity, back, "heart," chest, and "colic" ($n = 15$ patients). Tumors were discovered incidentally during routine physical examination or diagnostic radiographic studies performed for nonrelated reasons in nine patients. The female patients had a slightly shorter average duration of symptoms (37.6 months) than their male counterparts (41.2 months), but this finding was not statistically significant ($p = 0.850$). Interestingly, there was no significant difference in the mean duration of symptoms in patients with a syndrome or genetic disorder (mean 43.2 months) versus the other patients ($p = 0.843$), nor was there a significantly longer duration of symptoms for the patients with malignant tumors than the rest of the patients ($p = 0.284$).

TABLE 1. *Pheochromocytoma of the Adrenal Gland Scoring Scale (PASS)*

Feature	Score if present (no. of points assigned)
Large nests or diffuse growth (>10% of tumor volume)	2
Central (middle of large nests) or confluent tumor necrosis (not degenerative change)	2
High cellularity	2
Cellular monotony	2
Tumor cel spindling (even if focal)	2
Mitotic figures >3/10 HPF	2
Atypical mitotic figure(s)	2
Extension into adipose tissue	2
Vascular invasion	1
Capsular invasion	1
profound nuclear pleomorphism	1
Nuclear hyperchromasia	1
Total	**20**

HPF = high-power field.

Eleven patients had what was considered to be a nonsporadic tumor: an association with a syndrome known to include an increased incidence of adrenal pheochromocytoma. These patients include six male and five female patients (19–57 years of age, with a mean age at presentation of 35.5 years; significantly older than the patients with an associated syndrome who had malignant tumors [$p = 0.028$]). It is significant that five of these 11 patients had bilateral disease while none of the 39 remaining patients with benign tumors presented with bilateral tumors ($p < 0.001$). As would be expected with benign tumors, all syndrome-associated patients were alive ($n = 6$; mean 15.6 years) or had died without evidence of disease ($n = 5$; mean 11.4 years).

Laboratory values were available in 41 patients, all of whom had elevated levels in either their serum or urine of catecholamines, norepinephrine, epinephrine, metanephrine, dopamine, vanillylmandelic acid, or other metabolites. Without being able to review the laboratory printouts directly and with a variety of different techniques used to evaluate these catecholamines and their metabolites, no specific values are reported, but in 41 patients where values were available, elevation was seen in all cases.

Malignant

A summary of the clinical information on the patients in this series is provided in Table 3. The mean age at presentation for women was statistically significantly older than men (48.1 years and 42.6 years, respectively; $p = 0.033$). The mean age at presentation for the patients who had an associated syndrome or genetic abnormality

TABLE 2. *Immunohistochemical panel*

Antigen or antibody	Primary antibody	Company	Dilution	Antigen recovery
Chromogranin	mm	Boehringer Mannheim Biochemicals, Indianapolis, IN	1:3200	None
Synaptophysin	rp	Dako, Carpinteria, CA	Neat	Enzyme digestion
S-100 protein	rp	Dako	1:800	None
Serotonin	mm	Dako	1:25	None
Somatostatin	rp	Chemicon, Temecula, CA	1:2000	None
CD56 (NCAM)	mm	Zymed, San Francisco, CA	1:400	Microwave recovery
Cytokeratin (AE1/AE3 and CK1)	mm	Boehringer Mannheim Biochemicals and Dako	1:50 & 1:200	Enzyme digestion
Keratin 7	mm	Dako	1:200	Enzyme digestion
Keratin 20	mm	Dako	1:50	Enzyme digestion
Ki67	mm	Immunotech, Westbrook, ME	1:20	Microwave recovery
p53	mm	Dako	1:50	microwave recovery
Inhibin	mm	Serotec, Raleigh, NC	1:20	Microwave recovery

mm, mouse monoclonal; rp, rabbit polyclonal.

was young at 20.7 years ($p < 0.001$). At first thought it would seem that hypertension should be present in all patients with malignant pheochromocytomas (Table 3), but it is only present in 80% of patients with malignant pheochromocytomas. Tumors were discovered incidentally in four patients for similar reasons as those with benign tumors. The mean duration of symptoms of 8.9 months (Table 3) was much shorter than the duration of symptoms for patients with benign tumors ($p < 0.001$). The difference of average duration of symptoms between female and male patients was not statistically significant ($p = 0.571$), nor was the duration of symptoms in patients with a syndrome or genetic disorder statistically significant (mean 7.7 months; $p = 0.753$).

TABLE 3. *Clinical features of patients with pheochromocytomas*

	No.			
		Malignant histology		
Clinical characteristic	Benign (n = 50)	All cases (n = 50)	Benign clinically (n = 17)	Malignant clinically (n = 33)
Gender				
Women	20	23	6	17
Men	30	27	11	16
Age at presentation (years)				
Range	3–81	9–80	12–72	9–80
Mean	48.3	45.1	39.1	48.3
Females (mean)	49.5	48.1	31.2	54.1
Males (mean)	47.5	42.6	43.4	42.1
Patients with associated syndrome (mean)	35.5	20.7	17.7	23.7
Type of presentation*				
Hypertension	39	40	13	27
Diaphoresis, flushing, nausea, vomiting, headaches, palpitations	18	39	8	31
Pain (abdominal, chest, back, or ear)	15	17	6	11
Other (weakness, syncope, dizziness, anxiety, light headedness, shortness of breath, cough, regurgitation, diarrhea, bleeding [epistaxis, melena, hematuria, ejaculate], seizure disorder, weight loss, diabetes mellitus)	19	15	5	10
Asymptomatic (diagnostic evaluation)	9	4	2	2
Duration of symptoms (months)				
Range	1–240	0.5–60	1–24	0.5–60
Mean	40.0	8.9	9.1	8.8
Female (mean)	37.6	8.0	8.0	7.9
Male (mean)	41.2	9.6	9.5	9.7
Patients with a syndrome (mean)	43.2	7.7	10.0	5.3
Patients with syndrome	11	6	3	3
MEN or inherited forms of pheochromocytoma	8	4	2	2
Neurofibromatosis	1	1	1	0
von Hippel-Lindau syndrome	2	0	0	0
Turner's syndrome	0	1	0	1

* Patients may have presented with more than one symptom; therefore the numbers do not add up to the total number of patients.
MEN, multiple endocrine neoplasia.

Six patients had what was considered to be a nonsporadic tumor and included four male and two female patients (12–32 years of age, with a mean age a presentation of 20.7 years). It is significant that three of these six patients had bilateral disease with only one of 44 patients in the remaining malignant series ($p = 0.039$). Only one of these six patients died with disease after 17.6 years, whereas the remaining five are alive without evidence of disease (mean follow-up 19.9 years).

Similar to the benign tumors, laboratory values were available in 28 patients, all of whom had remarkably elevated levels in either their serum or urine of catecholamines, norepinephrine, epinephrine, metanephrine, dopamine, vanillylmandelic acid, or other metabolites. The levels were often elevated to >20 times the normal range for the analyte.

Radiographic Studies

Seventy-eight patients had one or more of a variety of imaging procedures performed before surgery, including abdominal roentgenogram, ultrasonography, computer tomography, magnetic resonance imaging, intravenous pyelogram, ^{131}I-meta-iodobenzylguanidine (MIBG) scintigraphy, and angiography. In general, a small to large, sharply defined suprarenal (adrenal) mass was identified, often displacing the kidney because of the mass' size. The lesion was described as heterogeneous or "in-homogeneous," demonstrating internal septations, fluid levels, and an increased signal or high proton density signal on T2-weighted image, interpreted as a hypervascular tumor. Angiography revealed a vascular tumor mass in the adrenal gland. Fifty cases in this clinical series were identified before 1985, before the widespread availability of the radiopharmaceutical and guanethidine analog ^{131}I MIBG. This radiocompound shows a remarkable affinity for the adrenal medullary tissue where it is taken up by neurotransmitter vesicles.[42] Whereas there were reports of "positive" imaging with this scintigraphic technique, there was not a sufficient number of these studies to be clinically meaningful in this series.

In many cases lytic bone lesions or "hypervascular" masses in the liver, lung, spleen, kidney, pancreas, and/or lymph nodes were noted and presumed by the radiologist to represent metastatic disease in the malignant cases. This finding was not identified in the benign cases.

Pathology

Macroscopic Findings

Benign. The majority of patients presented with unilateral disease (n = 45 patients), whereas five tumors were described as bilateral (Table 4). All of the bilateral tumors occurred in patients with syndromic association ($p < 0.001$). The tumors involved the right adrenal gland (n = 27 tumors) more frequently than the left adrenal gland (n = 18), a difference that reached statistical significance ($p < 0.001$), although there is no explanation for this difference. Overall, the mean tumor size was 6.5 cm with a mean tumor weight of 154 g. A separation between benign and malignant tumors by size or weight was attempted, but no matter what cutoff was used (50, 100, 200, or 500 g or 2, 4, 6, or 8 cm), no statistically significant difference could be generated, leading to the conclusion that increased size alone, although suggestive or worrisome for malignancy, was not an independently useful criterion (analysis of variance: weight, $p = 0.076$; size, $p = 0.219$; χ^2: weight, $p = 0.414$; size, $p = 0.200$). Furthermore, if confining the analysis to the malignant tumors alone, using a cutoff of 100 g, even though 29% of patients with tumors <100 g died with disease versus 47% of patients with tumors >100 g who died with disease, there was still no statistically significant difference ($p = 0.275$). Therefore, it seems that size is not helpful in predicting a difference in biologic behavior.

TABLE 4. *Macroscopic features of pheochromocytomas*

Macroscopic feature	Number			
		Malignant histology		
	Benign histology (n = 5)	All malignant cases	Benign clinically	Malignant clinically
Primary site				
Right	27	26	10	16
Left	18	20	6	14
Bilateral	5*	4*	1	3
Tumor size (cm)				
Range	1.3–18.0	2.8–18.0	3.0–12.5	2.8–18
Mean	6.5	7.9	6.6	8.6
Females (mean)	6.9	8.3	7.0	8.8
Males (mean)	6.2	7.5	6.3	8.4
Syndrome (mean)	4.8	4.8	5.7	4.0
Tumor weight (gm)				
Range	14–860	12–1320	12–653	1320
Mean	154.1	278	172	339
Females (mean)	132	344	158	437
Males (mean)	169	199	186	208
Syndrome (mean)	61.5	36.0	27.1	42.0

* All bilateral benign tumors and 3 of 4 malignant tumors occurred in syndrome-associated patients.

The tumors were described as encapsulated or well-circumscribed masses, with a variegated cut surface showing areas of hemorrhage or degenerative change. The tumors were soft, gray–tan to brown–red with areas of cystic degeneration. Compressed areas of bright yellow residual adrenal cortex were noted at the periphery of the tumor.

Malignant. The majority of patients presented with unilateral disease (n = 46 patients), with four tumors described as bilateral (Table 4). Three of the bilateral tumors occurred in patients with syndromic association,

TABLE 5. *Microscopic features of pheochromocytomas*

Microscopic feature	Number of cases with feature present			
		Malignant histology		
	Benign histology (n = 50)	All malignant cases (n = 50)	Benign clinically (n = 17)	Malignant clinically (n = 33)
Encapsulation	41 (82%)	47 (94%)	17 (100%)	30 (91%)
Vascular invasion	2 (4%)	29 (58%)	8 (47%)	21 (63%)
Capsular invasion	2 (4%)	32 (64%)	13 (76%)	19 (58%)
Extension into the periadrenal adipose tissue	0	13 (26%)	3 (18%)	10 (30%)
Architecture (no. of tumors with this dominant pattern)				
Zellballen	35 (70%)	5 (10%)	1 (6%)	4 (12%)
Large nests	9 (18%)	32 (64%)	11 (65%)	21 (63%)
Diffuse architecture	6 (12%)	13 (26%)	5 (29%)	8 (24%)
Cellularity				
Low	7 (14%)	0	0	0
Medium	43 (86%)	3 (6%)	1 (6%)	2 (6%)
High	0	47 (94%)	16 (94%)	31 (94%)
Cellular monotony	0	14 (28%)	2 (12%)	12 (36%)
Necrosis				
Pyknosis	4 (8%)	43 (86%)	15 (88%)	28 (85%)
Focal or central	0	15 (30%)	5 (29%)	10 (30%)
Confluent to diffuse	0	13 (26%)	4 (24%)	9 (27%)
Absent	46 (92%)	3 (6%)	1 (6%)	2 (6%)
Degenerative changes (including hemorrhage)	32 (64%)	34 (68%)	11 (65%)	23 (70%)
Fibrosis	8 (16%)	37 (74%)	12 (71%)	25 (76%)
Calcifications	0	4 (8%)	1 (6%)	3 (9%)
Spindle cell architecture				
<10% of tumor volume	6 (12%)	9 (18%)	4 (24%)	5 (15%)
>10% of the tumor volume	0	28 (56%)	9 (53%)	19 (58%)
Nuclear pleomorphism (moderate (benign) to severe)	22 (44%)	28 (56%)	9 (53%)	19 (58%)
Hyperchromasia	0	18 (36%)	8 (47%)	10 (30%)
Prominent, eosinophilic nucleoli	26 (52%)	25 (50%)	7 (41%)	18 (55%)
Intranuclear cytoplasmic inclusions	35 (70%)	31 (62%)	10 (59%)	21 (64%)
Eosinophilic globules	22 (44%)	22 (44%)	8 (47%)	14 (42%)
Tumor giant cells (size)	23 (46%)	30 (60%)	12 (71%)	18 (55%)
Mitotic figures				
Range	0–3	0–18	0–13	0–18
Mean	0.4	3	2.9	3.1
Atypical forms (no. of cases with atypical forms present)	0	19 (38%)	7 (41%)	12 (36%)
Inconspicuous or absent	32 (64%)	12 (24%)	3 (18%)	9 (27%)
Brown fat present	30 (60%)	31 (62)	10 (59%)	21 (64%)
Metastases (histology)				
Similar	n/a	17	n/a	17
More mature or "zellballen" architecture	n/a	5	n/a	5

a statistically significant finding ($p < 0.001$). The MPA also involved the right adrenal gland ($n = 26$ tumors) more frequently than the left adrenal gland ($n = 20$) but was not significant ($p = 0.453$). The mean tumor size was 7.9 cm with a mean tumor weight of 278 g. There were no statistically significant differences of the size or weight of tumors between the genders (weight, $p = 0.209$; size, $p = 0.409$).

The tumors were described as encapsulated or well circumscribed, nodular, lobulated, or bosselated masses, with a variegated cut surface showing mottled areas of hemorrhage and necrosis (Fig. 1). The tumors were soft, gray–tan to brown–red with areas of cystic degeneration and calcification. In a number of cases the tumor was described as fixed to the surrounding structures, occasionally infiltrating into the substance of the adrenal cortex and periadrenal adipose tissue. Small areas of yellow, residual adrenal cortex were noted at the periphery of the tumor.

Microscopic Findings

Benign. The tumors were well circumscribed and separated from the remaining adrenal cortex by a well-formed capsule in most cases ($n = 41$) (Table 5). Focal vascular invasion was identified in two cases. Capsular invasion was noted in two cases, while invasion into the periadrenal soft tissues was not noted in any tumors. The tumor cells were arranged in the characteristic "zellballen" architecture in all cases examined (Fig. 3), whereas nine cases demonstrated areas of large nest formation and six cases demonstrated diffuse architecture.

The tumors were of a low to medium cellularity with no tumors showing high cellularity. Tumor cell monotony was not identified. Pyknosis was noted in a few cases (n = 4 tumors), but focal, central, confluent, or diffuse necrosis was not appreciated. The majority of tumors showed areas of degenerative changes, including cyst formation, hemorrhage, hemosiderin-laden macrophages, and fibrosis. A focal spindle cell architecture was identified in six tumors but was limited to <10% of the tumor cell volume. Nuclear pleomorphism of a mild to moderate degree was noted in 22 cases. Tumor giant cells with many nuclei were seen in 23 tumors. Mitotic figures were inconspicuous or absent in the majority of cases, with a mean mitotic index of 0.6 mitosis per 10 HPF. Atypical mitotic forms (as defined in *Materials and Methods*) were not appreciated. When periadrenal adipose tissue was present, 30 of 34 cases demonstrated brown fat recognized by its finely reticulated or vacuolated cytoplasm and centrally placed nucleus. Although axiomatic, no tumors in this category developed lymph node or distant metastases during the follow-up period.

Malignant. The tumors were well circumscribed and distinctly separated from the remaining adrenal cortical parenchyma by a capsule. A well-formed tumor capsule was present in 47 cases, although the fibrous connective tissue capsule was thinned and attenuated in areas. Tumor cells were seen transgressing the capsule in 32 tumors (Fig. 2) or extending into the adjacent fibroadipose connective tissue. Vascular invasion was identified in 29 tumors. These vessels were identified within the fibrous capsule or beyond the tumor mass (Fig. 2) and occasionally identified macroscopically as the adrenal vein or vena cava.

There was extraordinary variation in histologic patterns both between tumors and within a given tumor. Even though histologic variability was predominant, the "zellballen" architecture with its prominent vascular supporting stroma could be found focally within each tumor, even if only constituting a small fraction of the overall tumor area. This nested growth was the dominant pattern in only five tumors (Table 5). The predominant histologic pattern was of large nests (n = 32 tumors) (Fig. 4). A "large nest" (described in *Materials and Methods*) was defined as three to four times the size of a "zellballen," or the size of the medullary paraganglia nests. Albeit a circuitous definition, the expanded size of a normal "cell ball" was very helpful in identifying malignant tumors. Moreover, areas of transition from small, evenly placed "zellballen" of pheochromocytes to the large nests (Fig. 4) raised one's index of suspicion for a malignant tumor. In 13 tumors a specific growth pattern was lost altogether and a diffuse pattern was the dominant finding, making up 60–100% of the tumor area (Table 5). Areas of transition between these patterns of growth, when present in the same tumor, were usually abrupt (Fig. 4). The rich vascular sustentacular supporting framework was easily identified in the areas of "cell ball" growth but became more difficult to identify in the large nests (although still present about the periphery of the large islands of tumor cells) and lost completely in the areas of diffuse growth.

Necrosis was assessed in three states: individual cell necrosis (pyknosis), focal necrosis, and confluent or diffuse necrosis (Table 5). Three tumors did not have any type of necrosis present. Pyknosis, sometimes difficult to assess if the specimen was not fixed well or if it was overstained, was present in 43 tumors, focal in four tumors, and otherwise easily identified throughout the other tumors. Focal necrosis was identified at the center of tumor cell nests (large nests) in 15 tumors (Fig. 4). The ghost outlines of tumor cells could be appreciated in the eosinophilic necrotic material. An additional 11 tumors demonstrated confluent or diffuse necrosis, in which the areas of necrosis had expanded to involve several cell nests (confluent) or had completely obliterated all tumor cells (diffuse necrosis). Necrosis was described as "tumor" necrosis, separate from areas of degenerative change. Degenerative changes were identified in 34 tumors and included cysts and lakes with proteinaceous material, hemorrhage, hemosiderin-laden macrophages, cholesterol clefts, stromal edema, noncoagulative necrosis (i.e., not tumor necrosis), inflammatory cells, focal fibrosis, and/or calcifications. Thirteen tumors had degenerative changes and no evidence of any tumor necrosis (focal or confluent), whereas five tumors had necrosis (two confluent and three focal) without degenerative changes. Thick, acellular bands of fibrosis were noted in 37 tumors, often accentuated around the periphery of the cell nests (whether "zellballen" or large nest type). Isolated calcifications were usually noted within areas of tumor necrosis.

The tumors were highly cellular in all cases except for three tumors, which were interpreted to have moderate cellularity. The cytoplasm was uniformly granular in all tumor cells, generally composed of fine, basophilic granules, but occasionally eosinophilic granules were also noted (n = 5 tumors). The cytoplasmic granules tended to vary in number based on the tumor cell size, such that gigantic tumor cells tended to have more granules than small tumor cells. Every effort was made to control for variation in staining technique by obtaining recuts, and for the most part, a basophilic tinctorial quality was present. In the areas of the tumor with large nests and diffuse architecture, it was common to see small, monotonous cells with indistinct cell membranes and a high nuclear-to-cytoplasmic ratio. Cellular monotony (n = 14) was more frequently noted in the areas of diffuse architecture than in other patterns of growth. Tumor cell spindling of >10% of the tumor volume was identified in 34 tumors (Table 5; Figs. 5 and 6, right). The cellular

TABLE 6. *Immunohistochemical panel results*

Antibody	No. of cases with positive reactions	
	Benign (n = 43)	Malignant (n = 33)
Chromogranin	43 (100%)	33 (100%)
Synaptophysin	41 (95.3%)	33 (100%)
S-100 protein	43 (100%)	18 (54.5%)
Serotonin	4 (9.3%)	1 (3.0%)
Somatostatin	4 (9.3%)	7 (21.2%)
CD56	39 (90.7%)	30 (90.9%)
Cytokeratin	5 (11.6%)	1 (3.0%)
Keratin 7	0	1 (F) (3.0%)
Keratin 20	0	1 (F) (3.0%)
Ki67	4—<1% 1—1–25%	18—<1% 3—1–25% 2—26–50%
p53	4—<1% 6—1–10% 12—11–90% 5—>90%	5—<1% 7—1–10% 5—11–90%
Inhibin	0	0

F, focal immunoreactivity.

elongation tended to be found within large nests, although detected anywhere in the tumor. The areas of spindling were usually composed of groups of cells arranged in streaming fascicles, rather than just being an isolated few cells.

Profound or severe nuclear pleomorphism included greatly enlarged nuclear size, irregular shape, and bizarre forms (Fig. 6, upper left). In a number of cases the tumor cells had a very high nuclear-to-cytoplasmic ratio, with small, monotonous cells identified (Table 5). Nuclear hyperchromasia was present in 17 tumors (Table 5), identified by complete opacification and heavy nuclear chromatin deposition. Prominent, enlarged (>4 μm in diameter), irregularly shaped, or eosinophilic-magenta nucleoli were observed (Fig. 6, upper left), as were intranuclear cytoplasmic inclusions and cytoplasmic eosinophilic hyaline globules (Table 5). Peculiar neoplastic giant cells were variably present throughout most of the tumors (n = 30) (Table 5), including multinucleated forms, but foreign body-type, Langerhans-type, and osteoclast-type giant cells were not present.

Mitotic figures were easily identified in the majority of tumors, although mitotic figures were inconspicuous (absent) in 12 tumors (Table 5). The mitotic index ranged from 1 to 18 mitoses per 10 HPF, with a mean of 3 mitoses per 10 HPF. Atypical mitotic forms were noted in 19 tumors, indicating that when mitotic figures were present, 50% of cases demonstrated atypical forms (Fig. 6, lower left).

The histology of the metastatic tumor deposits was available for review in 22 patients. The metastatic tumor was identical to the primary tumor in 17 patients, whereas in five patients the metastatic foci were different; the metastatic deposits had a typical "zellballen" architecture without the diffuse or large nest pattern. There was no difference in patient outcome based on the degree of "zellballen" architecture in the metastatic deposits ("zellballen" architecture, mean follow-up of 12.9 years; large nests, diffuse architecture, mean follow-up of 9.5 years; p = 0.537).

Special Procedures

All of the cases tested demonstrated positive pheochromocyte immunoreactivity with chromogranin (Fig. 7, left; Table 6) and nearly all cases with synaptophysin. It is well known that the sustentacular supporting framework cells are immunoreactive with S-100 protein (Fig. 7, right). The areas in which there was good "zellballen" architecture, S-100 protein-immunoreactive cells, could be seen in all cases. However, if areas of large nests and diffuse growth were examined, then there was only focal immunoreactivity in 18 of 33 tumors with this morphology (Fig. 8). Both the number of cells and the intensity of the reactivity decreased as the area of large nests and diffuse architecture increased (inverse relationship). It is important for us to stress that occasional S-100 protein-reactive cells could be identified in almost all cases, but the number of cells and the intensity of reactivity decreased remarkably as the size of the tumor nests enlarged or became diffuse. Nearly all cases tested also demonstrated strong, membrane immunoreactivity with CD56. There was no tumor cell immunoreactivity with inhibin.

Focal immunoreactivity was noted in a few cases for serotonin, somatostatin, cytokeratin, CK7, and CK20 (Table 6). Ki67 monoclonal antibodies, specifically MIB-1, react with antigens expressed in all active parts of the cell cycle.[3] Ki67 was remarkably variable in expression, ranging from essentially nonreactive up to 50% of the nuclei. However, 92.1% of both the benign and malignant cases tested were nonreactive or had <1% of the tumor nuclei immunoreactive, with only 7.9% demonstrating any significant reactivity (>10% of nuclei). All cases (n = 5) with >10% immunoreactivity occurred in malignant neoplasms, but this did not correlate with an adverse clinical outcome. p53 protein antibody reacts with the accumulated p53 protein in the cell nucleus, is usually increased in carcinomas, and consequently is frequently associated with a worse prognosis.[3] The results with p53 protein showed a similar variability as the Ki67, ranging from no reactivity to strong reactivity (in >90% of the cells' nuclei); 46.8% of cases demonstrated increased (>10%) nuclear reactivity. In general, the tumors

that were classified in the benign group expressed p53 to a greater degree than the malignant tumors. The patients whose tumors demonstrated increased p53 nuclear reactivity (n = 36 patients) had a mean follow-up of 12.8 years with three patients dying with disease (8.8%); the patients who did not have increased activity or were nonreactive (n = 31) had a mean follow-up of 11.4 years with eight patients dying of disease (19.0%), a difference that was not statistically significant (p = 0.072).

Clinical Therapy and Patient Outcome

All tumors were managed by surgery, even if only for the initial diagnostic biopsy, with preoperative adrenergic blockade to decrease intraoperative hypertensive episodes. The surgery included biopsy (n = 2); adrenalectomy (n = 69); adrenalectomy and lymph node dissection (n = 9); adrenalectomy and nephrectomy (n = 9); adrenalectomy, nephrectomy, and lymph node dissection (n = 2); adrenalectomy, nephrectomy, splenectomy, and lymph node dissection (n = 3); adrenalectomy, nephrectomy, and splenectomy (n = 2); adrenalectomy, lymph node dissection, and partial liver resection (n = 3); and adrenalectomy and lung resection (n = 1). In summary, 58 patients had adrenalectomy alone without additional surgery or adjuvant therapy, 31 patients had adrenalectomy with additional surgery, and 10 patients had surgery followed by adjuvant therapy (chemotherapy [n = 5] and/or radiation [n = 6]) (Table 7).

Benign

All patients with histologically benign neoplasms were managed by surgery alone (n = 50 patients) (Table 7). Most were managed by an adrenalectomy only (n = 39 patients), whereas six patients had a lymph node dissection and the remaining five patients had a nephrectomy (n = 4 patients) or partial pancreatectomy (n = 1 patient). In the patients who had bilateral tumors, bilateral adrenalectomies were performed (n = 5 patients). Although radiation and chemotherapy were not used to manage the pheochromocytomas, there was a subset of 12 patients who did have additional intervention. Six patients had additional surgery for benign tumors (retinal angioma, polycystic kidney and liver disease, parathyroid hyperplasia, and neurofibromas) related to an underlying syndrome. Seven patients had additional surgery for malignant tumors (medullary thyroid carcinoma, renal cell carcinoma) related to an underlying syndrome, and two patients had surgery for malignant tumors (colon carcinoma, ovarian carcinoma) unrelated to a particular or known syndrome. Furthermore, seven of these patients also had adjuvant chemotherapy for their malignant tumors (medullary carcinoma, n = 5; colon and ovarian carcinoma, n = 1 each, respectively). Of the group of 12 patients who had additional therapy, four patients died of metastatic medullary thyroid carcinoma (mean 13.1 years), one patient died of widely disseminated ovarian carcinoma (6.5 years), and one patient died of complications related to polycystic disease (4.6 years). The remaining six patients are all alive without evidence of any tumors (mean 15.9 years of follow-up). The mean survival for patients with bilateral tumors was 13.8 years (two alive with no evidence of disease [NED] and three dead with NED). The mean survival for patients with syndrome-associated pheochromocytoma was 13.7 years (six patients alive with NED and five patients dead with NED). In summary for the patients with benign tumors, there was an overall mean follow-up of 14.1 years, with 28 patients alive with NED (mean 16.7 years) and 22 patients who had died with NED (mean 10.7 years).

Malignant

The patients who had malignant tumors were managed by adrenalectomy alone, adrenalectomy with additional surgery, or followed by adjuvant therapy (chemotherapy [n = 5] and/or radiation [n = 6]). Interestingly, patients who were managed by surgery followed by either radiation or chemotherapy had a much longer survival (mean 9.0 years) despite 70% dying with disease compared with patients managed by surgery alone (mean 3.7 years) with 31% dying with disease. These data suggest that patients managed with surgery alone have a good prognosis but die with their disease sooner than patients managed with surgery and radiation. Patients who had an adrenalectomy only without any additional surgery or adjuvant therapy (n = 19) had an 89.5% survival with the two

TABLE 7. *Patient outcome based on treatment (all follow-up in years)*

	All cases		A, NED		A, D		D, NED		D, D	
	Benign	Malignant*	Benign	Malignant	Benign	Malignant	Benign	Malignant	Benign	Malignant
All cases	50 (14.1)	49 (8.3)	28 (16.7)	25 (11.7)	N/A	2 (1.0)	22 (10.7)	2 (7.2)	N/A	20 (5.6)
Adrenalectomy alone	39 (13.9)	19 (5.4)*	21 (16.5)	15 (6.1)	N/A	N/A	18 (10.9)	1 (7.4)	N/A	3 (0.8)
Adrenalectomy and other surgery (no adjuvant)	11 (14.7)	20 (11.5)	7 (17.6)	9 (20.1)	N/A	N/A	4 (9.6)	1 (6.9)	N/A	10 (4.3)
Any surgery followed by adjuvant therapy	N/A	10 (9.1)*	N/A	1 (19.0)	N/A	2 (1.0)	N/A	N/A	N/A	7 (9.0)

* Two patients had a biopsy only due to inoperability, but one patient had postoperative adjuvant therapy.

A, NEd, alive, no evidence of disease; A, D, alive, with disease; D, NED, dead, no evidence of disease; D, D, dead, with disseminated disease; N/A, non-applicable; adjuvant therapy includes radiation and/or chemotherapy.

patients who died with disease, dying in 4 and 16 months, respectively (Table 7). The patients with bilateral tumors had a mean follow-up of 19.7 years (one patient dead with disease [5.0 years] and three patients alive with NED). The patients with syndrome-associated pheochromocytomas had a mean follow-up of 20.3 years (one patient dead with disease [17.6 years] and five patients alive with NED).

To present the clinical outcome data in the most accurate form possible, two subcategories within the histologically malignant cases were created: clinically benign and clinically malignant (Table 8). The histologic features (presented in Table 5) are not statistically significantly different between these two clinical groups. By definition, there are no patients who died of their disease in the clinically benign group. Therefore, whereas one can retrospectively divide the patients into clinical behavior groups, they are indistinguishable from one another by histologic (and PASS) criteria in a prospective fashion.

Overall, 56% of patients are alive without evidence of disease or had died of unrelated causes without evidence of disease, with a mean follow-up of 11.0 years (Table 8). The remaining 44% of patients have evidence of disease: two patients are alive (0.6 and 1.3 years of follow-up, respectively) and 21 patients are dead with disease after surviving a mean of 5.2 years. It is of interest that no patients who developed lymph node metastasis solely died of their disease, but instead all were either alive (n = 7) or had died of unrelated causes (n = 1) without evidence of disease, with an average follow-up of 19.1 years. When widespread metastatic disease developed, 82.6% of patients died with disease, with a mean follow-up of 4.7 years. Likewise, it is interesting to note that when the patients survived (8.7%), they have a mean follow-up of 12.7 years: one patient developed liver and lung metastases, which were resected, and she has been followed for 19.1 years after irradium seeds were placed in the retroperitoneum; the other patient developed kidney and pancreatic metastases, which were resected, and she is alive with NED after 6.4 years. These results yielded an overall raw 5-year survival rate of 54% with only a 36% 5-year disease-free survival. Nine patients are alive without evidence of disease but have not yet been followed for 5 years. Twelve patients had evidence of local (periadrenal or para-aortic) lymph node metastasis at the time of initial presentation: six patients (58.3%) are alive with no evidence of disease (mean follow-up, 16.7 years), one patient died with NED at 7.4 years, and five patients died with disseminated disease (mean survival 6.7 years).

When metastatic disease was present, the lymph nodes and skeletal system were the most frequent sites of involvement (n = 17 each, respectively), followed by liver and lung (n = 16 each, respectively), brain (n = 3), peritoneum (n = 3), stomach (n = 2), and one each to the diaphragm, pleura, spleen, pancreas, ileum, and kidney. However, only five patients had widely metastatic disease at the time of initial presentation, and all of these patients had evidence of disease at the last follow-up; four patients were dead with disease (mean survival 0.5 year) and one patient is alive with disease at 0.6 year.

DISCUSSION

Pheochromocytomas are uncommon tumors of the sympathochromaffin system; although the histologic diagnosis is relatively easy, the distinction of a benign from a malignant neoplasm based on histologic grounds alone has not been easy. Most paraganglia neoplasms (i.e., pheochromocytomas) occur in the adrenal gland,[13,14,49] with these catecholamine-producing tumors generating a well-defined clinical presentation of secondary episodic, labile, and/or paroxysmal hypertension. The true incidence of MPA is difficult to determine, but

TABLE 8. *Overall patient outcome (follow-up in years)*

	A, NED			A, D			D, NED			D, D		
		Malignant histology			Malignant histology			Malignant histology			Malignant histology	
	Benign histology	Benign clinically	Malignant clinically	Benign histology	Benign clinically	Malignant clinically	Benign histology	Benign clinically	Malignant clinically	Benign histology	Benign clinically	Malignant clinically
All cases	28	16	9	0	0	2	22	1	1	0	0	21
Survival range (yr)	10.5–21.7	1.3–27.8	1.8–26.6	N/A	N/A	1.6–2.3	0.3–18.7	6.9	7.4	N/A	N/A	1.7–24.1
Survival mean (yr)	16.7	8.6	20.0	N/A	N/A	2.0	10.7	6.9	7.4	N/A	N/A	5.2
With lymph node metastases only*	0	N/A	7	0	N/A	N/A	0	N/A	1	0	N/A	0
Survival mean (yr)	N/A	N/A	21.8	N/A	N/A	N/A	N/A	N/A	7.4	N/A	N/A	N/A
With widespread metastases*	0	N/A	2	0	N/A	2	0	N/A	N/A	0	N/A	21
Survival mean (yr)	N/A	N/A	13.7	N/A	N/A	2.0	N/A	N/A	N/A	N/A	N/A	5.2

* Not all cases reviewed developed lymph node or widespread metastases, and so the numbers in these groups do not necessarily equal the overall survival.

A, NED, alive, no evidence of disease; A, D, alive, with disease; D, NED, dead, no evidence of disease; D, D, dead, with disseminated disease; N/A, non-applicable.

based on this series, MPA comprise about 6.5% of all benign or malignant tumors of the adrenal gland, a value similar to that reported in the literature.[14,15,18,32]

There is no gender predilection for pheochromocytomas as a whole or when examining BPA or MPA.[14,18,21,24,27,32,44,45] The mean age at presentation ranges from 32 to 61 years[14,18,21,24,27,28,32,33,44,45]; in this clinical series the mean was 46.7 years (BPA 48.3 years; MPA 45.1 years). As would be expected, the mean age at presentation for patients with a syndromic association was younger (33.9 years).[27] In this clinical series 17% of patients' tumors presented in a nonsporadic setting, higher than the 8–12% usually reported in other series,[14,18,21,49] but this finding is perhaps related to the referral nature of the Armed Forces Institute of Pathology. However, the vast majority of cases in this clinical series were sporadic. Syndrome-associated patients had significantly more bilateral tumors than sporadic patients (88.9% versus 11.2%; $p < 0.001$).[26,48] Therefore, it is safe to postulate that if bilateral disease is present, an added effort to exclude a familial or syndrome association is warranted.[18,21,35,48] In general, the pheochromocytomas associated with syndromes behave in a benign fashion (only one patient [5.9%] in this clinical series died with disease after 17.6 years),[48] although there are some exceptions.[30,51]

A variety of the symptoms and signs for pheochromocytoma are nonspecific, but hypertension (sustained, episodic, paroxysmal, and/or labile) was usually identified in most patients.[14,18,21,27,28,32,49] Asymptomatic presentation in this clinical series (13%) was seen more frequently in patients with BPA than patients with MPA. The overall mean duration of symptoms for this clinical series is 23.0 months, whereas patients with MPA had a much shorter duration of symptoms (mean 8.9 months) than patients with benign tumors (mean 40.0 months) ($p < 0.001$).

For some unknown reason, pheochromocytomas, whether benign or malignant, seem to be more commonly found in the right adrenal gland, a finding also noted in other clinical series.[14,18,27,32,35,44] MPA tend to be nodular to bosselated with areas of necrosis and hemorrhage, unlike BPA. There was no difference in patient outcome based on size or weight, irrespective of a benign or malignant designation. A number of different size and weight cutoffs (not a statistically sound approach) were used to be absolutely certain that an increased weight and/or large size did not have an independent prognostic significance (weight, $p = 0.076$; size, $p = 0.219$), especially because this finding is in sharp contrast to previously reported series.[14,28,32]

Pheochromocytomas are made up of a dual cell population. The main cell is the chief cell or pheochromocyte (chromaffin) cell, which can be detected on routine hematoxylin and eosin-stained slides. The second population is the sustentacular cell, which is thought to be a supporting cell, similar to the glial cells in the central nervous system. They are slender cells, with thin wisps of cytoplasm encompassing the chief cells, accurately identifiable only with special studies.[13,14,35,45]

The definition of malignancy for pheochromocytoma has been and still is exceedingly difficult to define on histologic criteria alone. According to the literature, the most rigorous definition of malignancy requires that metastases should be present at a site where chromaffin tissue is not otherwise found, thereby excluding the possibility of misclassifying multicentric primary lesions as metastases or the development of locoregional recurrence.[2,9,19,24,25,32,33,35,44] The most common sites of metastatic disease are to the axial skeleton, lymph nodes, liver, lung, and kidney,[14,21,27,28,30,32–34,44,45,51] a finding consistent with this clinical study, although other uncommon sites of metastasis have been reported (such as pericardium, brain, and spleen).[32,51] Furthermore, when a patient presents with recurrent signs and symptoms of pheochromocytoma (elevated catecholamines), a distinction between local recurrence, an asynchronous second primary tumor, or metastatic disease needs to be made. This distinction can be fraught with difficulty, especially because the biochemical findings are identical. To date, there have been no absolute clinical, macroscopic, microscopic, biochemical, radiographic, or cytometric indicators of malignancy.[14,24,32,41]

It is important to stress that no single histologic feature is diagnostic for malignancy. Similar to malignancies of other organs, and especially of other endocrine organs, a constellation of histomorphologic features must be taken into consideration before a diagnosis of malignancy can be made. In the purest sense possible (and in keeping with the tradition presented in the literature), the development of metastatic disease (lymph node and/or other organ site) was taken to be indicative of a malignant primary tumor. With this endpoint in mind, a weighted scale (PASS) was applied to a variety of histomorphologic features, which seemed to be different in quality, quantity, or incidence between tumors that behaved in an entirely benign fashion and those that demonstrated metastatic disease. These features included vascular invasion (1 point), capsular invasion (1 point), extension into the periadrenal adipose tissue (2 points), large nest size or diffuse architecture (2 points), presence of focal or confluent necrosis (2 points), high cellularity (2 points), tumor cell spindling (2 points), cellular monotony (2 points), >3 mitoses per 10 HPF (2 points), presence of atypical mitotic figures (2 points), profound nuclear pleomorphism (1 point), and increased tumor cell hyperchromasia (1 point). These weightings were based on the suggested sensitivity of these features to detect "malignant" tumors that had developed documented metastatic disease. Special studies were not included in

this PASS because they may not be available in all settings, add to the turnaround time and to the overall cost, and may not always contribute to the final diagnosis.

It is important in a scoring system not to undercall a potentially malignant tumor, thereby depriving a patient of the maximum possible benefit of close clinical follow-up, additional surgery, or the application of adjuvant therapies. Based on an application of the weighted scoring scale (PASS), all patients who had developed documented metastatic disease in this clinical study had a total score of ≥4 points. The patient survival data were separated into three basic groups: a benign histology with a benign clinical outcome (n = 50), a malignant histology with a benign clinical outcome (n = 17), and a malignant histology with a malignant clinical outcome (n = 33). All of the patients with a malignant histology had a PASS of ≥4, irrespective of outcome. This means that of the entire group of 100 patients in this study, 50 tumors were classified as histologically malignant, but 17 of these patients have not developed metastatic disease (eight of whom have not yet been followed for 5 years). Therefore, although no documented metastases were identified in these 17 patients, the tumors had all of the features of morphologic malignancy identical to those in the neoplasms that metastasized. It is of great interest that the PASS yielded only three tumors with a combined score of >15 (maximum score of 20), two patients have not yet developed metastatic disease (both are alive, one at 3.0 years and the other at 27.8 years), and one died of disseminated disease at 2.9 years. Therefore, although this scoring scale will hopefully provide a valuable adjunct in the prospective management of patients, a combined score of ≥4 in no way guarantees the development of metastatic disease or suggests that the patient will die from their disease. The corollary to this statement also deserves proclamation: a combined score of ≤3 does not guarantee that a patient will not go on to develop metastatic disease at some point in the future.

A total score of ≥4 (Table 1), when applied to this clinical series, correctly identified all pheochromocytomas, which ultimately developed documented metastatic disease ($p < 0.001$, regression analysis). In this clinical series of 100 patients with adrenal pheochromocytomas, a combined score of ≤3 was found in 50 patients, none of whom developed any metastatic disease with a mean follow-up of 14.1 years. If the patients who are alive are reviewed separately, they have a mean follow-up of 16.8 years. This would suggest that if metastatic disease is still to develop, it would be a considerable length of time after the initial presentation. There have been reports of metastatic disease developing many years after the primary tumor[18,46] and in apparently histologically BPA in up to 6.5% of cases,[18,21,46] but the histologic presentation is not enumerated in detail and thus this author cannot determine if the tumors would be classified as benign or malignant using the PASS. In either case, based on the prolonged evolution of pheochromocytoma, it is imperative to maintain lifelong clinical follow-up of patients with malignant pheochromocytomas by both laboratory or radiologic investigation in addition to following the patients' symptoms at initial presentation to see if they recur.[18,19,21,24,32,34,35,44,45]

Although the PASS is an accurate histologic method of separating potentially aggressive tumors from those that do not behave in an aggressive fashion, it does not imply a "cookbook" recipe for success when applying these criteria. They must always be applied in an individual patient clinical context, allowing for differences in individual patient treatment. There will inevitably be a case that will defy any classification system as suggested by the two cases in this clinical series with a high PASS total. I think that the PASS can help to predict which tumors may portend a more aggressive clinical course. Therefore, it is my contention that malignancy should not be based solely on the development of metastases but augmented by the use of the PASS. The microscopic features of the PASS appear to predict with confidence which neoplasms are more likely to behave in a clinically aggressive manner ($p < 0.001$). Again, there is a degree of subjectivity in the application of the criteria (such as hyperchromasia or profound nuclear pleomorphism). Therefore, the scaled nature of this score was implemented to try to accentuate features that may be more important when present. The PASS will need to be applied to a larger number of cases to determine its clinical utility and validity because any one of the histologic features documented in malignant cases (including vascular or capsular invasion, tumor cell spindling, cellular hyperchromatism, necrosis, and increased mitotic activity) has been described in tumors that did not go on to recur or develop metastatic disease.[11,14,24,28,31,46,49] The other histologic features catalogued are of interest and value in the diagnosis of pheochromocytoma in general but not in distinguishing between BPA and MPA.

There is a lack of an intermediate category in this classification (undetermined malignant potential, low-grade malignant tumor, uncertain malignant potential), which may be a potential drawback to its application in the clinical setting. However, if any one of the specific criteria examined is deleted or given a different weighting, bona fide malignant cases that developed documented metastatic disease would not be correctly identified. Furthermore, the treatment options for such an "intermediate" category are vague and not well developed, whereas the management of benign or malignant tumors is well inculcated into the literature.

Although special studies were not included in the PASS, the S-100 protein reaction was of interest in the diagnosis of MPA. S-100 protein immunoreactivity was identified in both the nucleus and cytoplasm of the sus-

tentacular cells surrounding the chromaffin cells.[13,45] The intensity of the staining within the sustentacular cells, and occasionally in the chromaffin cells, is variable not only within a case but also between cases. In many cases heavy, intense staining of the sustentacular cells was present, only to become weak or absent in areas of large nest formation or diffuse growth pattern. There was a remarkable decrease in the immunohistochemical reactivity of S-100 protein in malignant cases (six of 13 did not react with S-100 protein at all).[13,28,45] It is important to add that S-100 protein-positive cells were noted in metastatic deposits (especially in areas of "cell ball" growth, whether in lymph nodes or other organs), suggesting that the sustentacular cells had metastatic potential along with the chromaffin cells and are an integral part of the tumor. Therefore, based on the findings in this study and those of other authors, the absence of sustentacular cells in pheochromocytoma is indicative of a greater potential for malignant behavior.[13,45]

Flow cytometric nuclear DNA analysis was not performed in this series, but this technique has yielded mixed results, including diploid, tetraploid, and aneuploid populations present in both benign as well as clinically malignant pheochromocytomas. It has been suggested that cases that have disease progression all had abnormal DNA findings and all who died had abnormal DNA findings. No patients with a diploid population of cells died of their disease. Therefore, although a tetraploid or aneuploid tumor population does not confirm a malignant diagnosis, it may suggest a more aggressive biologic behavior.[1,5,20,22,35,41] There have also been studies to suggest that *ret* proto-oncogene is overexpressed in pheochromocytomas but does not have rearrangements.[40]

The usual prognosis of malignant pheochromocytoma is poor, not only related to metastatic disease but also to heart failure related to excess catecholamines in the circulation, although highly variable in a number of patients. In this clinical series there was a 36% 5-year disease-free survival or a 54% raw 5-year survival, similar to the 44–57% 5-year survival reported by others.[18,24,32–35,44,47] The beneficial effect of adjuvant therapy is limited in extent to a decrease in the amount of hormone effect (partial or complete) and a reduction in the size of the tumor (either partial or complete). But overall, chemotherapy (including, although not limited to, a combination of cyclophosphamide, vincristine, and dacarbazine) and radiation therapy (including treatment with ^{131}I-MIBG) are only of benefit in palliation by ameliorating the hypertensive effects of the excess catecholamines and not in cure or complete remission.[1,4,10,28,29,32,35,37,39,43,44,47,52] In a few cases the treatment with radiation or chemotherapy has induced a hypertensive crisis if the patients were not adequately adrenergically blocked.[1,52] Whereas surgery remains the stalwart of therapy, it is important to note that ^{131}I-MIBG must be accumulated in the tumor cells to have a therapeutic effect. If the metastatic or recurrent tumor deposits do not accumulate ^{131}I-MIBG in the cells, then this treatment modality does not yield any patient benefit. However, if there is uptake, then subjective improvement of the hormonal systems, lowering of the blood pressure, and pain relief can generally be expected, although complete remission has yet to be achieved.[1,4,10,44]

A number of different tumors need to be considered in the differential diagnosis, although in general benign versus malignant pheochromocytoma is the more difficult distinction. An adrenal cortical carcinoma generally will not have the same architectural pattern. The cells tend to contain eosinophilic cytoplasm and have vesicular or microvacuolated cytoplasm and more mitotic activity. Metastatic tumors account for more neoplasms in the adrenal gland than primaries and may mimic a pheochromocytoma, but immunophenotypic differences will help to exclude metastatic disease. Although a malignant pheochromocytoma is thought to be a tumor of the paraganglia cells, if the malignancy is composed almost exclusively of a spindle cell proliferation, a malignant transformation of the sustentacular cells should be considered. This type of tumor would almost certainly mimic a malignant peripheral nerve sheath tumor in architectural and cytomorphologic features while also exhibiting S-100 protein immunohistochemical staining.[6,16,26] In this clinical series there was no evidence to support such a malignant transformation, although I do not doubt the theoretical possibility.

I think that the application of the PASS can benefit the clinical management decision tree for patients with adrenal pheochromocytomas, thereby reserving the more aggressive treatments for tumors that have a higher probability of behaving in a biologically aggressive fashion. Widespread application of this scaled score with appropriate clinical follow-up will help to validate these findings. □

Acknowledgments

The author thanks Tara Kelly-Baker, PhD, for her statistical analysis.

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