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Adrenal Cortical Neoplasms in the Pediatric Population

A Clinicopathologic and Immunophenotypic Analysis of 83 Patients

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Adrenal cortical neoplasms in pediatric patients (<20 years) are rare. The clinical manifestations and biologic behavior of these lesions can be quite distinct from their histologically similar counterparts in the adult population, making pathologic criteria for distinguishing benign from malignant tumors equivocal. We undertook a study of 83 adrenal cortical neoplasms to determine if adult clinical and histologic features can be applied to pediatric patients in an outcome-based analysis. Most of the patients (50 girls and 33 boys) presented with hormone-related symptoms present for a mean of 6.8 months. The tumors ranged in size from 2 to 20 cm (mean 8.8 cm). Histologic parameters examined included capsular and/or vascular invasion, extraadrenal soft tissue extension, growth pattern, cellularity, necrosis, cytoplasmic eosinophilia, nuclear pleomorphism, nuclear-to-cytoplasmic ratio, prominent nucleoli, mitotic figures, atypical mitotic figures, bands of fibrosis, and calcifications. Immunophenotypically, there was reactivity with inhibin, vimentin, CK5, and focally with p53 and Ki-67. All patients underwent adrenalectomy, and 20 patients received adjuvant therapy. All patients with tumors classified as adenomas (n = 9) were alive, without evidence of disease (mean 14.7 years), whereas 21 patients with carcinomas had died with disease (mean 2.4 years). Only 31% of histologically malignant tumors behaved in a clinically malignant fashion. Features associated with an increased probability of a malignant clinical behavior included tumor weight (>400 g), tumor size (>10.5 cm), vena cava invasion, capsular and/or vascular invasion, extension into periadrenal soft tissue, confluent necrosis, severe nuclear atypia, >15 mitotic figures/20 high power fields, and the presence of atypical mitotic figures. Vena cava invasion, necrosis, and increased mitotic activity (>15 mitotic figures/20 high power fields) independently suggest malignant clinical behavior in multivariate analysis.

Key Words: Adrenal—Neoplasm—Carcinoma—Pediatric—Prognosis.

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Adrenal cortical neoplasms in the pediatric population are rare, with adrenal cortical carcinomas (ACC) accounting for the majority of the tumors. It is estimated that approximately 25 cases of pediatric adrenal cortical neoplasms occur in the United States per year, of which approximately 75% are ACC.[4,15,22,42] Of greatest clinical importance, pediatric adrenal cortical neoplasms appear to behave differently than histologically similar tumors in the adult population. Pathologic criteria for malignancy in the adult population are well established in the literature.[10,37,39,40] In contrast, definitive pathologic criteria for malignancy in adrenal cortical neoplasms in the pediatric age group remain uncertain. This is due, in part, to the rarity of tumors in this age group as well as to the lack of clinicopathologic correlation of the patients' outcomes. It is often the case that a child with an adrenal cortical neoplasm with apparently poor prognostic features (based on adult criteria) will have a good clinical outcome. With these prefatory remarks in mind, we undertook this comprehensive study of adrenal cortical neoplasms in children to determine if there are any pathologic parameters that may be predictive of biologic outcome in this patient population. We evaluated these tumors based on gross and microscopic features, immunohistochemical reactivity, clinical behavior, and treatment outcomes.

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MATERIALS AND METHODS

The records of 96 pediatric patients with tumors diagnosed as adrenal cortical adenoma, adrenal cortical neoplasm of uncertain malignant potential, adrenal cortical adenoma with atypical features, adrenal cortical neoplasm, or adrenal cortical carcinoma were identified in the files of the Endocrine Registry at the Armed Forces Institute of Pathology from 1965 to 1997. These 96 cases were identified in a review of 4772 all benign or malignant primary adrenal neoplasms seen in consultation during this same period. However, 13 patients were excluded from further consideration because of at least one of the following reasons: 1) paraffin blocks were unavailable for additional sections; 2) only a core needle biopsy or fine needle aspiration was received for review; and/or 3) the originally submitted case did not have sufficient demographic information supplied from which to obtain adequate and complete follow-up information. Therefore, the remaining 83 patients with adrenal cortical neoplasms constitute the subject of this study based upon complete follow-up information and sufficient material to obtain a definitive or "diagnostic" hematoxylin and eosin-stained slide to confirm a diagnosis. Inclusion in this study required the presence of an adrenal cortical neoplasm and excluded adrenal medullary (pheochromocytoma, neuroblastoma, ganglioneuroma) and metastatic tumors. Seventy-six cases were obtained from civilian sources, including university medical centers, pediatric hospitals, and foreign contributors, and eight cases were received from military hospitals.

Materials within the Institute's files were supplemented by a review of the patients' demographics (gender and age); symptoms at presentation (including duration); and past history (specifically, any syndrome association such as hemihypertrophy, café au lait spots, Beckwith-Wiedemann syndrome). 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 included tumor size and stage, treatment methods used, and current patient and disease status. It is important to add that we are a tertiary pathology review center, conducting a retrospective review of these patients and we did not treat the patients. 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.

The macroscopic pathologic features of the tumors were obtained from the gross descriptions of the individual contributing pathologists as reported in their surgical pathology reports. Hematoxylin and eosin-stained slides from all cases were thoroughly reviewed by the three investigators. A comprehensive list of macroscopic and histologic observations were noted and recorded as follows: tumor size (greatest dimension in centimeters); tumor weight (in grams); location (right or left); circumscription; nodularity/bosselation (Fig. 1); encapsulation; capsular invasion (into or through the capsule with stromal reaction; Fig. 2); vascular invasion (sinusoidal [without smooth muscle], venous [with smooth muscle; Fig. 2] or vena cava); invasion into periadrenal soft tissue or adjacent organs; the presence or absence of fibrous bands; cytoplasmic eosinophilia (Fig. 3); the presence or absence of tumor cell necrosis (focal to confluent, coagulative necrosis of tumor cells, not just degenerative changes) (Fig. 4); tumor cellularity (low, high; Fig. 5); growth pattern (diffuse/solid, trabecular, nested/alveolar; Fig. 5); cellular monotony; nuclear pleomorphism (mild [grade I], moderate [grade II], severe [grade III-IV]) (Fig. 4); prominent nucleoli; nuclear hyperchromasia; increased nuclear-to-cytoplasmic (N/C) ratio; mitotic figures (number of mitotic figures per 20 high power fields [HPF; magnification at ×40x with a ×10 objective lens using an Olympus BX40 microscope]); atypical mitotic figures (presence or absence, defined by abnormal chromosome spread, tripolar or quadripolar forms, circular forms or indescribably bizarre) (Fig. 6); and the presence of retrogressive changes, including calcifications and hemorrhage.

Immunophenotypic analysis was performed in all cases with suitable material by a standardized Envision method using 4-μm-thick, formalin fixed, paraffin-embedded sections. Table 1 documents the pertinent, commercially available immunohistochemical antibody panel used. The analysis was performed on a single representative block for each primary tumor. 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. Heat-induced epitope retrieval was performed, as required, by using formalin-fixed, paraffin-embedded tissue treated with a buffered citric acid solution, pH 6.0 (Citra, Dako Corporation, Carpinteria, CA, USA), and heated for 20 minutes in a steamer. Following this, 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 absent to weak (0–1+), moderate (2+ to 3+) and strong (4+) staining, and the fraction of positive cells was determined by separating them into four groups: <10%, 11–50%, 51–90%, and >90%.

Initially, cases were designated as adenoma or carcinoma based on histologic features adapted from the established histologic criteria used in adults (Table

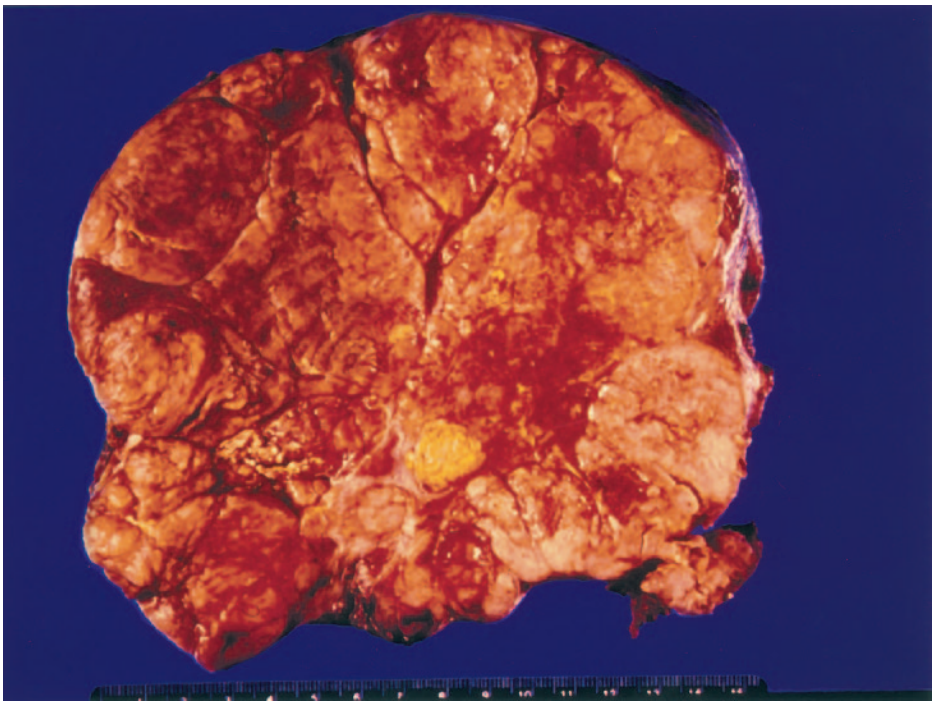

FIG. 1. A large tumor with gross hemorrhage and necrosis, demonstrating multilobularity and bosselation with nodular growth. Residual adrenal cortex is not identified.

2).[10,37,39,40] We required the presence of at least four of the listed criteria (Table 2) for a definitive diagnosis of ACC. Follow-up information was then obtained blindly, without knowing the pathologic designation of the individual cases. Subsequent to obtaining the follow-up information, cases were divided into a "good outcome" group, defined by cases in which the patients had no evidence of recurrent or persistent disease or death from disease, and a "poor outcome" group, defined by recurrent or persistent disease or death from disease. The cases were then divided into three groups combining clinical outcome with pathologic features: group A, clinically benign and histologically benign; group B, clinically benign and histologically malignant; and group

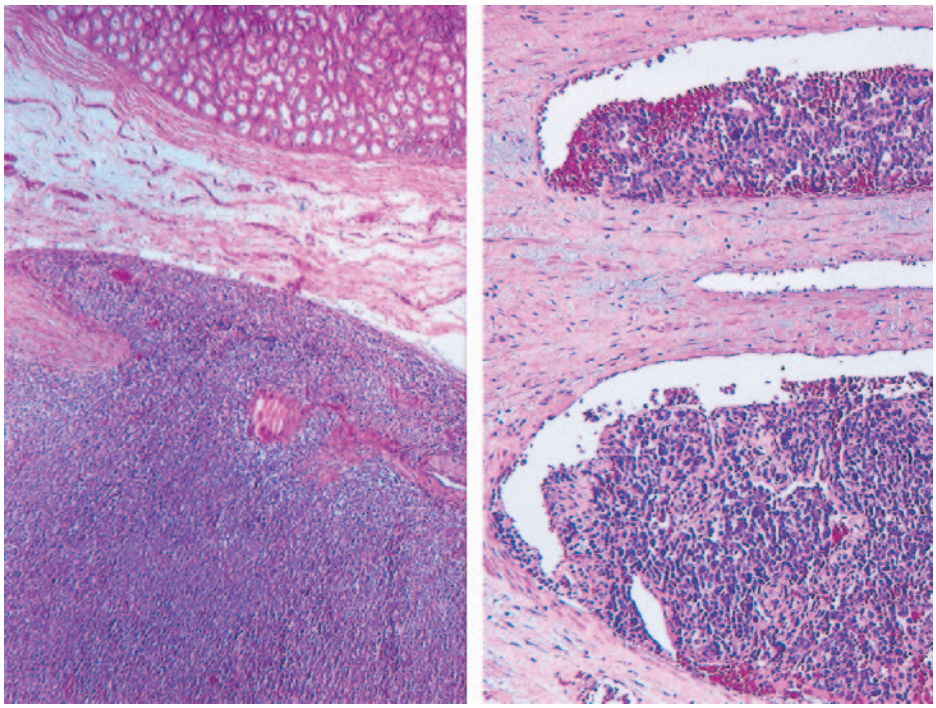

FIG. 2. Capsular invasion with extension into the periadrenal soft tissue by the neoplastic cells (left); uninvolved kidney is noted at the top of the image. Vascular invasion is demonstrated by tumor cells attached to large muscle walled vessels (right), a feature that proved to have prognostic significance.

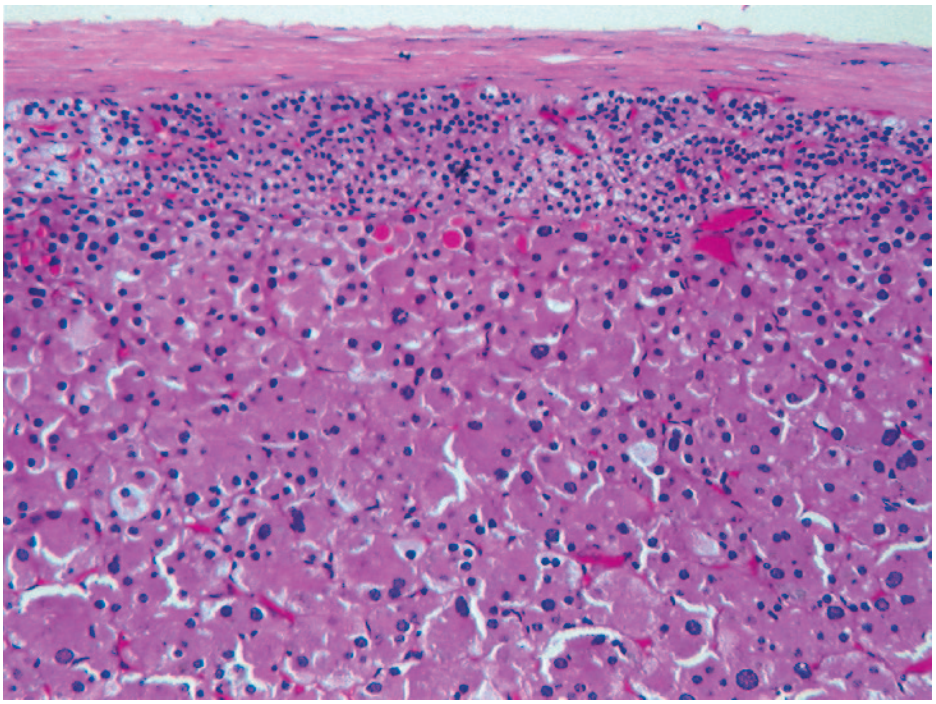

FIG. 3. A benign adrenal cortical adenoma with compressed adrenal cortex overlying large cells with abundant eosinophilic cytoplasm.

C, clinically malignant and histologically malignant. Statistical analysis was performed using this grouping system. A general adrenal cortical neoplasm staging was used based on a proposed system by Lack[14]: stage I, tumor without invasion; stage II, local invasion but without direct extension into surrounding organs; stage III, lymph node metastasis (regional); and stage IV, disseminated disease.

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

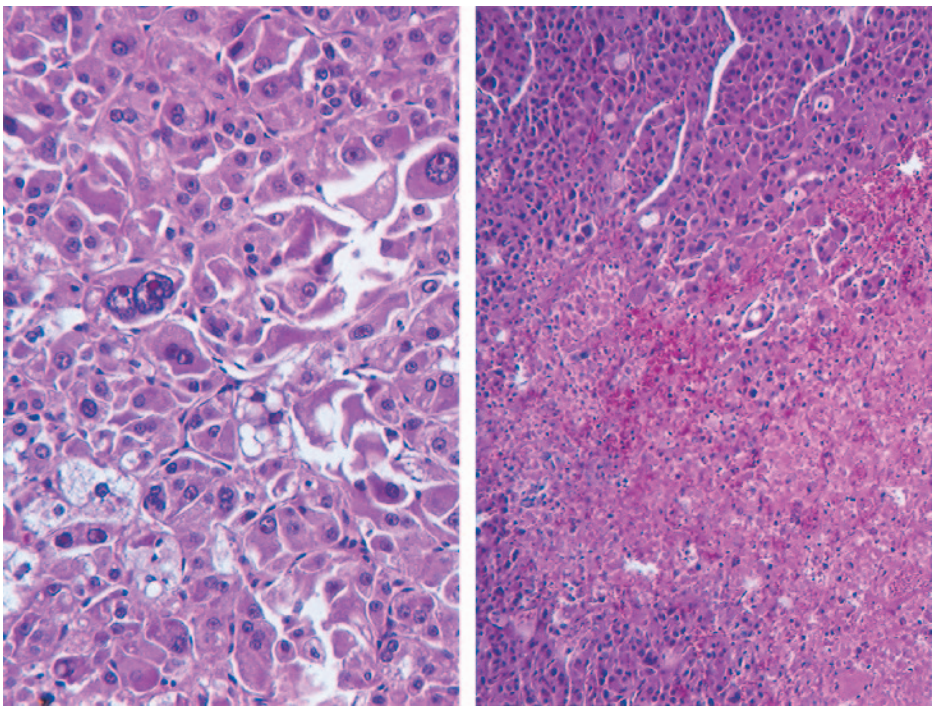

FIG. 4. Significant nuclear pleomorphism with an increased nuclear-to-cytoplasmic ratio could be seen in lipid-depleted, eosinophilic cells, whereas finely vacuolated clear cytoplasm was noted in a minority of cells (adrenal cortical adenoma, left). Confluent tumor necrosis in a clinically malignant case, group C (right).

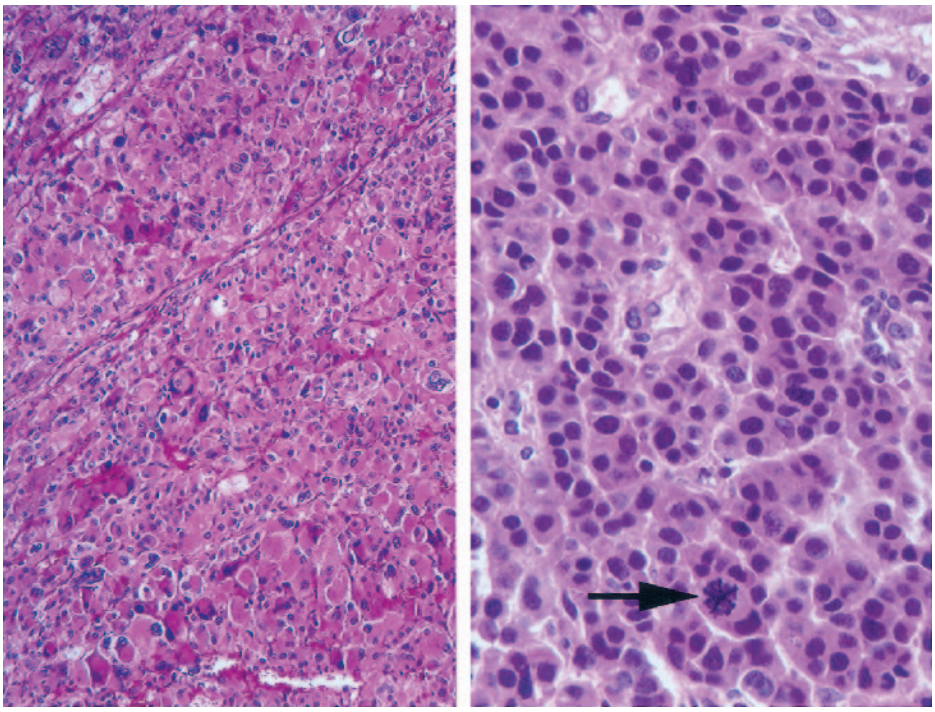

FIG. 5. Highly cellular tumor with the compact eosinophilic cells arranged in a broad trabecular architecture with moderate to severe nuclear pleomorphism (left); in another case, atypical mitotic figures are easily identified (right).

correlation coefficients were generated to measure the strength of the association. The variables that significantly predicted malignant outcome in the univariate analyses were entered into a single model. A Forward Stepwise model-building test procedure was used. After four analytic steps, the factors that significantly predicted malignant outcome were tested in a main effects model (with no interacts). Confidence intervals of 95% were generated for all positive findings. The alpha level was set at $p < 0.05$. All analyses were conducted using Statistical Package for the Social Sciences (SPSS) software (version 8.0 for PC; Chicago, IL, USA).

Our review of adrenal cortical neoplasms in children in the English literature was based on a MEDLINE

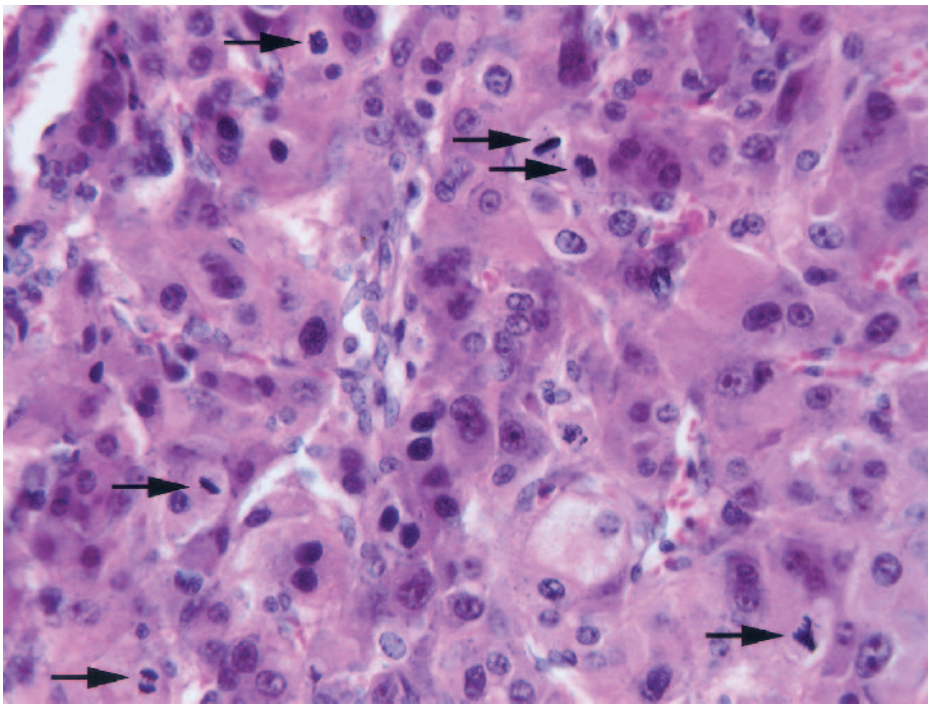

FIG. 6. Adrenal cortical carcinoma from group B shows increased numbers of mitotic figures, with six mitotic figures noted in this single high power field (arrows). An atypical mitotic figure is noted (bottom right).

TABLE 1. *Immunohistochemical panel*

Antigen or antibody	Primary antibody	Company	Dilution	Cellular conditioning
Cytokeratin	mm	Boehringer Mannheim Biochemicals,	1:50	Enzyme digestion
(AE1/AE3 and CK1)	mm	Indianapolis, IN, and Dako, Carpinteria, CA	1:200	
Keratin 7	mm	Dako	1:200	Enzyme digestion
Keratin 20	mm	Dako	1:50	Enzyme digestion
CK5/6	mm	Boehringer Mannheim Biochemicals	1:60	Steam
CAM5.2	mm	Ventana, Tucson, AZ	1:100	Enzyme digestion
Epithelial membrane antigen	mm	Dako	1:100	Enzyme digestion
Carcinoembryonic antigen	rp	Dako	1:800	Enzyme digestion
Inhibin	mm	Serotec, Raleigh, NC	1:20	Steam
Vimentin	mm	Ventana	1:400	N/A
Chromogranin	mm	Boehringer Mannheim Biochemicals	1:3200	N/A
Synaptophysin	rp	Dako	Neat	Enzyme digestion
S-100 protein	rp	Dako	1:800	N/A
Estrogen receptor	mm	Dako	1:40	Steam
Androgen receptor	mm	BioGenex, San Ramon, CA	1:100	Steam
bcl-2	mm	Dako	1:200	Steam
Melan A	mm	Novacastra, New Castle, UK	1:40	Enzyme digestion
Ki67	mm	Immunotech, Westbrook, ME	1:20	Steam
p53	mm	Dako	1:50	Steam

mm, mouse monoclonal; rp, rabbit polyclonal. N/A, not applicable.

search from 1966 to 2002, with a few specific earlier articles included for balance and background. Because of the large number of single case reports, we refined our review to reports with at least five adrenal cortical neoplasms (adenomas or carcinomas) in pediatric patients, that included incidence, diagnostic guidelines, clinical management, and treatment information.

RESULTS

The 83 patients included in our study represented 1.8% of the 4772 all benign and malignant primary adrenal gland neoplasms diagnosed during the period 1965–1997. Furthermore, these 83 patients represent 0.13% of the 60,732 benign and malignant primary neoplasms diagnosed in pediatric patients during the same reference period.

The clinical features of the 83 patients studies are summarized in Table 3. There were 50 girls and 33 boys, ranging in age from 4 months to 19 years (mean 7.6 years; median 4 years). The female patients had a slightly older mean age at presentation than the male patients (8.7 years vs 5.5 years). There did seem to be a biphasic age distribution, with 53% of the cases (n = 44) presenting in children under the age of 5 and 37% of the cases (n = 31) presenting in those over the age of 10 years. Only 10% of the cases (n = 8) presented in children between the ages of 5 and 10 years. The vast majority of patients (n = 67) presented with signs or symptoms of endocrine dysfunction, including precocious puberty with virilization, Cushing's syndrome, feminization, or a combination thereof. Most patients presented with clinical evidence of virilization, whereas 12 patients presented with a mixed endocrine syndrome (11 virilization and Cushing's syndrome; 1 feminization and Cushing's syndrome). Those that did not show clinical evidence of endocrine dysfunction presented with such symptoms as abdominal mass, abdominal pain, and fever. One patient

TABLE 2. *Histologic criteria for malignancy of adrenocortical neoplasms in adults (adapted)*[10,37,39,40]

Diffuse growth pattern predominates with loss of normal architecture (>30% of the tumor mass)
Eosinophilic cytoplasm (>30% of the tumor composed of lipid-depleted cells with compact, eosinophilic cytoplasm)
Vascular invasion
Capsular invasion
Tumor cell necrosis (confluent)
Broad fibrous bands (>1 HPF in diameter)
Mitotic rate (>2/10 HPF)
Atypical mitotic figures (abnormal spindles or chromosome distribution)
Moderate to marked cellular pleomorphism, nuclear atypia (grade II–III)
Moderate to marked hyperchromasia
Prominent nucleoli

HPF, high-power field (400×).

TABLE 3. *Clinical features of 83 pediatric patients with adrenal cortical neoplasms*

Clinical characteristic	No.				
			Malignant histology		
	All cases examined (n = 83)	Benign histology (n = 9)	All cases (n = 74)	Benign clinically (n = 51)	Malignant clinically (n = 23)
Gender					
Female	50	6	44	29	15
Male	33	3	30	22	8
Age at presentation (y)					
Mean	7.6	10.6	7.1	5.4	10.9
Girls (mean)	8.7	11.7	8.3	6.4	12.0
Boys (mean)	5.5	8.3	5.2	4.0	8.7
Type of presentation*					
Virilization	38	6	32	24	8
Feminization†	1	0	1	1	0
Cushing's syndrome	17	3	14	8	6
Mixed	12	0	12	11	1
Other (mass, pain, fever, volvulus)	24	0	24	12	12
Asymptomatic (diagnostic evaluation)	3	0	3	3	0
Duration of symptoms (mo)					
Mean	6.8	12.4	6.2	6.6	5.2
Female (mean)	6.8	13.3	6.1	5.9	6.6
Male (mean)	6.8	11.3	6.3	7.8	2.7

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

† The single patient with feminization was part of a mixed endocrine syndrome.

presented at 19 years of age with volvulus, and the adrenal mass was discovered incidentally at the time of surgery. Three patients were completely asymptomatic, and the adrenal tumor was discovered incidentally during unrelated diagnostic evaluation. Mean duration of symptoms for the group as a whole was 6.8 months, without any gender differences.

A number of congenital associations have been reported in children diagnosed with adrenal cortical neoplasms, including hemihypertrophy, urinary tract abnormalities, adrenal cytomegaly, and Beckwith-Wiedemann syndrome, and cancer family syndromes such as Li-Fraumeni and SBLA syndrome (S, sarcoma; B, breast and brain neoplasms; L, leukemia, laryngeal and lung carcinoma; A, ACC).[18] In our study there were two patients with hemihypertrophy, one case on the same side as the tumor and the other on the contralateral side. Four patients were reported to have café au lait spots, and one patient had horseshoe kidney. There were no cases associated with Beckwith-Wiedemann syndrome, Li-Fraumeni syndrome, or SBLA syndrome.

A number of various radiographic diagnostic methods were used in the initial evaluation of the patients including plain films, computed tomography scans, magnetic resonance imaging, intravenous pyelograms, and arteriography. The vast majority of studies reported an adrenal or suprarenal mass with varying radiographic qualities (heterogeneous, calcified, low density, well delineated). Laboratory investigation was also undertaken in the majority of patients and demonstrated the following. Of the 38 patients with evidence of virilization, 26 had elevated testosterone and/or DHEA levels. Of the 17 patients with clinical signs of Cushing's syndrome, 10 had evidence of elevated serum or urinary ketosteroids, hydroxycorticosteroids, and/or cortisol/ACTH levels. Of the 11 patients with mixed virilization and Cushing's syndrome, six had elevated levels of both testosterone/DHEA and corticosteroids (urinary and/or serum), four had elevated levels of corticosteroids only, and one did not show laboratory evidence of endocrine dysfunction. The single patient with mixed feminization and Cushing's syndrome did not have any reported significant laboratory abnormalities.

Using the established pathologic criteria for malignant adrenal cortical neoplasms in adults,[10,37,39,40] we were able to categorize the tumors as adrenal cortical adenomas (n = 9) and ACCs (n = 74). After obtaining follow-up, three groups were identified: group A, clinically benign and pathologically benign (n = 9); group B, clinically benign and pathologically malignant (n = 51); and group C, clinically malignant and pathologically malignant (n = 23). All cases that were originally diagnosed as "adenoma" based on the adult criteria did turn out to have a good clinical outcome (n = 9) and compose group A. Group B (n = 51) is composed of cases that were diagnosed pathologically as carcinoma but had good outcome (as defined in *Materials and Methods*), and group C is composed of cases that were diagnosed pathologically as carcinoma and had a poor outcome (as defined in the *Materials and Methods*).

Group A: Clinically Benign and Pathologically Benign (n = 9)

Clinical Features

There were six girls and three boys with a mean age of 10.6 years (Table 3). The female patients had a slightly older age at presentation (mean 11.7 years) than the male patients (mean 8.3 years). The majority of cases presented with clinical evidence of virilization (n = 6), and three cases presented with evidence of Cushing's syndrome. No congenital associations were noted in this group.

Macroscopic Features

The mean tumor size was 4.7 cm (range 2–10 cm) with a mean tumor weight of 82 g (range 11–210 g) (Table 4). The tumors were described as well circumscribed or encapsulated with occasional areas of hemorrhage. There was no evidence of extension of the tumor into adjacent soft tissue or other organs in this patient group, nor was there evidence of invasion into the vena cava.

Microscopic Features

These tumors were circumscribed and encapsulated in all cases with no evidence of capsular or vascular invasion (Table 5). The majority of cases (n = 8) had >30% of the tumor composed of a diffuse or solid growth pattern. Cases varied from low (n = 4) to medium cellularity (n = 5) (Fig. 3). Three cases had histologic evidence of fibrosis or broad fibrous bands traversing the tumor, and one case displayed focal tumor necrosis. Overall, the tumors demonstrated mild to moderate nuclear pleomorphism, with one case showing significant pleomorphism (Fig. 4). Two cases displayed cellular monotony with little variation in cell shape or size. The majority of cases had a normal N/C ratio, although two cases had a moderate N/C ratio; a high N/C ratio was not appreciated in any case in this group. Interestingly, eight of the nine cases were composed predominantly of cells that were lipid-depleted with a compact eosinophilic cytoplasm (>30% of the tumor) (Figs. 3 and 4). No nuclear hyperchromasia was noted, but five tumors showed prominent nucleoli. The mean number of mitotic figures per 20 HPF was 1.8 with a range of 0–11. A single atypical mitotic figure was observed in one tumor. Microcalcifications were not seen.

Treatment and Outcome

All patients in this group were managed by adrenalectomy alone (Table 6). All patients had a good clinical outcome and were alive without evidence of disease at the last follow-up (mean 14.7 years).

Group B: Clinically Benign and Pathologically Malignant (n = 51)

Clinical Features

There were 29 girls and 22 boys in this group with a mean age of 5.4 years (Table 3). Again, the female pa-

TABLE 4. *Macroscopic features of 83 pediatric patients with adrenal cortical neoplasms*

	No.				
			Malignant histology		
Macroscopic feature	All cases examined (n = 83)	Benign histology (n = 9)	All cases (n = 74)	Benign clinically (n = 51)	Malignant clinically (n = 23)
Primary site					
Right	42	6	36	24	12
Left	39	3	36	25	11
Unknown	2	0	2	2	0
Tumor size (cm)					
Mean	8.8	4.7	9.2	7.8	12.2
Girls (mean)	9.3	5.5	9.9	8.1	13.3
Boys (mean)	7.7	3.3	8.2	7.5	10.1
Tumor weight (gm)					
Mean	378	82	391	268	631
Girls (mean)	399	90	442	295	673
Boys (mean)	270	40	285	224	484
Vena cava invasion	8	0	8	2	6
Extension into periadrenal soft tissues and/or adjacent organs	15	0	15	6	9
T stage (pathologic staging)					
T1	15	6	9	8	1
T2	53	3	50	37	13
T3	11	0	11	4	7
T4	4	0	4	2	2

TABLE 5. *Microscopic features of 83 pediatric patients with adrenal cortical neoplasms*

Microscopic feature	No.				
			Malignant histology		
	All cases examined (n = 83)	Benign histology (n = 9)	All cases (n = 74)	Benign clinically (n = 51)	Malignant clinically (n = 23)
Vascular invasion	32	0	32	20	12
Capsular invasion	39	0	39	24	15
Architecture (no. of tumors with this pattern)*					
Alveolar	12	2	10	8	2
Diffuse/solid	71	8	63	44	19
Trabecular	45	2	43	27	16
Cellularity					
Low	5	4	1	1	0
Medium	36	5	31	22	9
High	42	0	42	28	14
Dense, acellular bands of fibrosis	42	3	39	30	9
Necrosis					
Absent	30	8	22	21	1
Present	53	1	52	30	22
Nuclear pleomorphism					
Severe	13	1	12	10	2
Cellular monotony	32	2	30	19	11
High nuclear to cytoplasmic ratio	44	0	44	29	15
Eosinophilic, lipid-depleted cytoplasm	82	8	74	51	23
Hyperchromasia	22	0	22	14	8
Prominent nucleoli	46	5	41	29	12
Mitotic figures (per 20 high-power fields)					
Range	0–59	0–11	0–59	0–59	0–50
Mean	11.5	1.8	12.7	8.7	21.7
Atypical forms (# of cases with atypical forms)	43	1	42	24	18
Calcifications	30	0	30	22	8

* Tumors generally had more than one pattern of growth present; therefore, the numbers do not add up to the total number of patients.

tients had a slightly older age at presentation (mean 6.4 years) than the male patients (mean 4.0 years). Virilization (n = 24) was the most frequent finding with Cushing's syndrome (n = 8) and a mixed endocrine picture of virilization and Cushing's syndrome in 11 patients. One patient presented with mixed endocrine features of feminization and Cushing's syndrome. Twelve patients presented with other clinical features such as mass, fever, pain, or other nonspecific complaints. Three patients were asymptomatic, and tumors were discovered incidentally on workup for unrelated reasons. Two patients were noted to have hemihypertrophy, both on the right side, three had café au lait spots, and one patient had horseshoe kidney.

TABLE 6. *Patient outcome based on treatment (all follow up in years)*

	All patients			Histologically benign (n = 9)		Histologically malignant (n = 74)					
				Clinically benign (n = 9)		Clinically benign (n = 51)			Clinically malignant (n = 23)		
	A, NED	D, NED	D, D	A, NED	D, NED	A, NED	D, NED	D, D	A, NED	D, NED	D, D
All patients	58 (17.0)	4 (4.2)	21 (2.4)	9 (14.7)	0	47 (18)	4 (4.2)	0	2 (9.6)	0	21 (2.3)
Treatment type											
Surgery alone (n = 55)	49 (17.1)	4 (4.2)	2 (0.6)	9 (14.7)	0	39 (17.9)	4 (4.2)	0	1 (10.0)	0	2 (0.6)
Surgery/chemo (n = 20)	5 (14.2)	0	15 (2.0)	0	0	4 (15.4)	0	0	1 (9.2)	0	15 (2.0)
Surgery/radiation (n = 3)	3 (22.2)	0	0	0	0	3 (22.2)	0	0	0	0	0
Surgery/combination (n = 5)	1 (8.0)	0	4 (4.1)	0	0	1 (8.0)	0	0	0	0	4 (4.1)
T stage											
T1 (n = 15)	13 (17.4)	1 (0.1)	1 (1.1)	6 (18.8)	0	7 (16.3)	1 (.03)		0	0	1 (1.1)
T2 (n = 53)	39 (16.9)	3 (5.7)	11 (2.9)	3 (6.7)	0	34 (18.3)	3 (5.7)	0	2 (9.6)	0	11 (2.9)
T3 (n = 11)	4 (15.3)	0	7 (1.5)	0	0	4 (15.3)	0	0	0	0	7 (1.5)
T4 (n = 4)	2 (18.4)	0	2 (1.9)	0	0	2 (18.4)	0	0	0	0	2 (1.9)

A, NED, alive, no evidence of disease; D, NED, dead, no evidence of disease; D, D, dead, with disseminated disease; chemo, chemotherapy; combination, includes radiation and chemotherapy.

Macroscopic Features

The mean tumor size was 7.8 cm (range 2–20 cm) with a mean tumor weight of 268 g (range 7–2413 gm) (Table 4). The majority of cases were described as well circumscribed and/or encapsulated (n = 38). Gross evidence of hemorrhage or necrosis was described in 24 of the cases. Sixteen of the cases were described as multilobulated or bosselated, divided by fibrous bands. Six cases in this group had extension into the adjacent soft tissues (periadrenal fat, Gerota's fascia) or organs (kidney, diaphragm). Two cases had invasion into the vena cava.

Microscopic Features

The tumors in this group were also encapsulated and circumscribed for the most part (n = 45) (Table 5). Twenty-four cases displayed capsular invasion and 20 cases had evidence of vascular invasion (17 sinusoidal invasion and 7 venous invasion [4 cases had both sinusoidal and venous invasion]). Most cases displayed a diffuse/solid growth pattern (n = 44) in >30% of the tumor. Twenty-seven cases had focal trabecular growth (Fig. 5), whereas only an occasional tumor (n = 8) displayed areas of nested growth. Cellularity varied from medium (n = 22) to high (n = 28), with only one case having low cellularity. Broad fibrous bands and/or acellular fibrosis was present in the majority of cases (n = 30). Similarly, tumor necrosis was present in many cases (n = 30). Overall, most cases exhibited moderate to severe nuclear pleomorphism (n = 23 moderate, n = 10 severe; Fig. 5) with an additional 18 cases showing only mild nuclear pleomorphism. Nineteen (37%) of the cases showed an overall monotonous growth. An increased N/C ratio was present in the overwhelming majority of cases, with 29 cases demonstrating a high N/C ratio and 21 cases demonstrating a moderately increased N/C ratio. A lipid-depleted, eosinophilic cytoplasm in >30% of the tumor cells was the rule in this group, with all cases showing loss of a lipid-rich cytoplasm; the cases varied from having a granular eosinophilic cytoplasm to a more dense, compact eosinophilic cytoplasm. Hyperchromasia was present in only 14 cases, whereas prominent nucleoli were noted in 29 cases. The mean number of mitotic figures per 20 HPF was 8.7 with a range of 0–59 mitoses; 24 cases had atypical mitotic figures (Figs. 5 and 6). Microcalcifications were recognized in 22 cases.

Treatment and Outcome

The majority of these patients were managed by adrenalectomy alone (n = 43); however, four patients did have adjuvant chemotherapy at the time of diagnosis (one of these patients had partial hepatectomy [for concurrent hepatoblastoma] in addition to adrenalectomy as part of their original surgical procedure) and three patients had adjuvant radiation therapy (Table 6). One patient had combination adjuvant therapy (chemotherapy and radiation therapy) at the time of diagnosis. By definition, all patients in this group had good outcome without recurrence or development of metastatic disease at any point during the follow-up period. The one patient who had concurrent hepatoblastoma was alive and well with no evidence of either neoplasm at the last follow-up (15 years).

Group C: Clinically Malignant and Pathologically Malignant (n = 23)

Clinical Features

There were 15 girls and 8 boys in this group with a mean age of 10.9 years (Table 3). The female patients were slightly older at presentation (mean 12.0 years) than the male patients (mean 8.7 years). Eight patients presented with signs of a virilizing tumor, whereas six patients presented with clinical evidence of Cushing's syndrome. One patient presented with a mixed endocrine picture with a combination of virilizing and Cushingoid features. Twelve patients presented with other clinical features such as mass, fever, and pain, and one patient presented with volvulus. No patients in this group were noted to have hemihypertrophy or urinary tract abnormalities, but one patient did have multiple café au lait spots.

Macroscopic Features

The mean tumor size was 12.2 cm (range 5–20 cm) and the mean tumor weight was 631 g (range 24–2260 g) (Table 4). Thirteen of the cases were described as well circumscribed or encapsulated. Fifteen of the cases had gross evidence of hemorrhage and/or necrosis, and 10 cases were described as multilobular, bosselated, and/or nodular (Fig. 1). Nine cases had evidence of extension into adjacent soft tissue or organs, primarily kidney, with invasion of the vena cava in six cases.

Microscopic Features

Only about 50% of these lesions were noted to be encapsulated (n = 12) and/or well circumscribed (n = 13) (Table 5). Fifteen of the cases showed evidence of capsular invasion (Fig. 2). Twelve cases had vascular invasion (Fig. 2), either sinusoidal or venous or both. The majority of cases (n = 19) showed a diffuse/solid or trabecular growth pattern in >30% of the tumor. Increased cellularity (medium [n = 9] to high [n = 14]) was seen in all cases. Broad fibrous bands or acellular fibrosis was prominent in only a minority of cases (n =

9), whereas tumor necrosis was present in an overwhelmingly majority (n = 22). Only 12 cases had moderate to severe nuclear pleomorphism (n = 10 moderate, n = 2 severe), whereas all cases had a moderate to high N/C ratio. Areas of cellular monotony were present in approximately 50% of the cases (n = 11). All cases were composed of lipid-depleted cells, which contained compact eosinophilic cytoplasm in >30% of the tumor volume. Eight (35%) of the cases showed nuclear hyperchromasia. Prominent nucleoli were seen in 12 (53%) cases. The mean number of mitotic figures per 20 HPF was 21.7 with a range of 0–50 mitoses; 18 cases had atypical mitotic figures. Eight cases revealed focal microcalcifications.

Treatment and Outcome

Only three of these cases were managed by surgery alone. The remaining 20 patients were treated with adjuvant therapy consisting of either combined chemotherapy and radiation therapy (n = 4) or chemotherapy alone (n = 16); no patient in this group was treated with radiation only (Table 6). All patients in this group by definition had poor outcome, either developing recurrent (n = 15) or metastatic (n = 21) disease and/or dying from disease (n = 21). Two of the patients had recurrent disease but were alive with no evidence of disease at the time of the last follow-up (10.0 and 9.2 years, respectively). Metastases to local lymph nodes (periadrenal, aortic, and celiac; n = 5) was similar in frequency to distant lymph nodes (n = 6). Of the 21 patients who died of their disease, 20 had evidence of metastasis. Overwhelmingly, the most common sites of metastatic disease were lung (n = 17), liver (n = 16), gastrointestinal tract (n = 10), and bone (n = 9), with other less frequent sites, including brain (n = 1) and gynecologic tract (n = 1).

Long-term Outcomes

The overall patient outcome was 74.7%. The mean follow-up was 14.7, 16.7, and 3.0 years, respectively, for group A, B, and C patients. The average follow-up for histologically malignant cases (groups B and C combined) was 12.4 years. The overall 5- and 10-year raw survival rates were 68.6% and 60.2%, respectively, with overall 5- and 10-year disease-free survival rates of 63.9% and 59.0%, respectively. This suggests that once patients survived beyond 5 years, their chance of dying from disease diminished precipitously.

Immunohistochemical Results

Thirty cases had suitable material for immunohistochemical evaluation. The results are summarized in Table 7. All cases (100%) showed diffuse and strong reactivity with antibodies to the α-subunit of inhibin. CK5/CK6 was positive in 75% of cases tested, whereas other keratins were only sporadically immunoreactive. It has been suggested that the presence of p53 and Ki-67 expression in adrenal cortical neoplasms may indicate a worse prognosis.[23,29,38] None of the adenomas (group A) showed Ki-67 or p53 expression. Variable expression for Ki-67 and p53 was present in groups B and C. However, the presence of Ki-67 or p53 expression did not differ statistically between groups B and C; thus, in our study, expression of Ki-67 and/or p53 did not appear to predict poor patient outcome.

TABLE 7. *Immunohistochemical panel results*

Antibody	No. of cases with positive reactions (%)	
	Histologically benign	Histologically malignant
Inhibin	100	100
Vimentin	100	84
CK5/6	100	72
Keratin 7	0	27
Keratin 20	0	10
Cytokeratin	0	13
CAM5.2	0	10
Epithelial membrane antigen	0	0
Carcinoembryonic antigen	67	17
Melan A	67	30
Synaptophysin	0	4
Estrogen receptor	0	3
Androgen receptor	0	3
bcl-2	0	8
Chromogranin	0	0
S-100 protein	0	0
Ki67	0	37
p53	0	50

Statistical Summary

Clinical Features

A statistically significant difference in the mean age at presentation between patients in groups B and C was noted (older for group C patients), but no other clinical features analyzed proved to be of statistical significance in predicting outcome.

Macroscopic Features

Tumor size and tumor weight correlated with patient outcome: the 95% confidence interval for tumor size was 12.2 ± 1.7 cm; thus, a tumor size >10.5 cm suggests a poor outcome ($p < 0.001$). Correspondingly, the 95% confidence interval for tumor weight was 630 ± 227 g; thus, a tumor weight >400 g suggests a poor outcome ($p = 0.015$). Extension into periadrenal soft tissues and/or adjacent organs ($p = 0.004$) and invasion into the

vena cava ($p = 0.004$) were macroscopic variables that predicted a poor prognosis. In multivariate analysis, vena caval invasion alone greatly increased the probability of a malignant outcome ($p = 0.023$), whereas the other factors were not independent predictors of a poor outcome.

Microscopic Features

Microscopic features suggestive of a poor biologic outcome with univariate analysis included the following: presence of vascular [venous] invasion ($p = 0.001$); capsular invasion ($p = 0.043$); tumor necrosis ($p = 0.004$); increased mitotic activity >15/20 HPF ($p = 0.001$); and the presence of atypical mitotic figures ($p = 0.001$). In multivariate analysis, only the presence of necrosis alone or the presence of increased mitotic activity alone (based on >15/20 HPF) increased the likelihood of malignant behavior ($p = 0.05$ and $p = 0.002$, respectively). The presence of nuclear hyperchromasia and microcalcifications, although not statistically significant, was only seen in histologically malignant cases. It is worth noting, in light of the adult criteria, that the following features had no significant impact on patient outcome: hormone production ($p = 0.07$), severe nuclear pleomorphism ($p = 0.47$), broad fibrous bands ($p = 0.19$), and lipid-poor, compact eosinophilic cytoplasm ($p = 0.80$).

DISCUSSION

The rarity of adrenal cortical neoplasms in children makes coordinated and directed research efforts difficult, leading to a paucity of information upon which to base pathologic interpretation and hampering the therapeutic decision-making process. The established criteria[10,37,39,40] for distinguishing benign from malignant adrenal cortical neoplasms in the adult population have not been helpful in predicting the biologic behavior of such neoplasms in the pediatric population. In the distant past, most children with adrenal cortical tumors had a poor prognosis, perhaps because they would die following surgery of acute adrenal cortical insufficiency. Now, with the availability of pharmacologic replacement of cortisol, patients with histologically malignant-appearing tumors do well, often better than their adult counterparts.[9] In further support of this claim, 51 of 74 (69%) patients with histologically malignant-appearing tumors in this clinical series had a benign clinical course without recurrence or death from disease. This raises the question: Are there any criteria that one can use to predict the biologic behavior of adrenal cortical neoplasms in pediatric patients to facilitate therapeutic management decisions?

In contrast to the adult population, tumors in children more often are hormonally functional with clinical evidence of virilization, Cushing's syndrome, mixed endocrine syndromes, and rarely feminization or Conn's syndrome.[5,13,15,16,24,28] Further, feminizing tumors in adults are fatal, a fate not shared in pediatric patients (the patient in this clinical series is alive without evidence of disease), although death from tumor in this population has been reported.[6] Whereas a functional tumor may be indicative of poor outcome in adult patients,[6,20] the same cannot be extrapolated to the pediatric population.

Although girls are affected more frequently than boys,[5,15,16,21,24,28] gender did not prove to be a statistically significant predictor of an adverse patient outcome. There appears to be a biphasic age distribution[11]: an "infantile" group (<5 years) and an "adolescent" group (>10 years). The patients with a poor clinical outcome tend to fall into the "adolescent" group,[11,30,43] although age alone does not seem to independently predict clinical outcome.

With increasing tumor size and weight, the likelihood of malignant behavior increases,[1,2,21,24,43] to the point that some have suggested tumors >500 g are invariably malignant.[3] Although in this series, a tumor weight of >400 g was statistically predictive of poor patient outcome ($p = 0.01$), it did not prove to be an independent prognostic factor and so cannot be used as the sole criterion for malignancy.[11,17] There were a few tumors that weighed >500 g and yet enjoyed a good clinical outcome (the largest tumor in this clinical series [2413 g] occurred in a patient with a clinically benign course), whereas tumors as small as 24 g yielded a malignant clinical outcome. Our proposed cutoff of 400 g is higher than others,[28] suggesting that higher weight is *more* predictive of patient outcome. Furthermore, tumor size (as measured linearly in centimeters), directly related to weight, follows a similar predictive course.[5,43] Tumors >10.5 cm had a statistically worse outcome than those of smaller size. Again, however, size alone was not an independent prognostic factor: a 20-cm tumor (along with 12 other tumors >10.5 cm) occurred in patients who had a good clinical outcome.

Other features that we found to be statistically predictive of patient outcome were extension into periadrenal soft tissues and/or adjacent organs and invasion into the vena cava. No pediatric patient studies explicitly examined these features prognostically, although in adults these features place the tumor into an advanced T stage (T3 or T4).[19,34] In our analysis, these factors appear to be predictive of worse patient outcome ($p = 0.01$), with the presence of vena cava invasion independently predicting a poor patient outcome ($p = 0.01$). A separate staging system for childhood adrenocortical tumors has been proposed that differs from the adult population.[27,31] Because of the referral nature of our consultation practice, we could not reliably apply the complex standards to separate tumors into benign, intermediate, and high-stage

disease. Suffice it to say that increased stage does correlate with a poor clinical outcome (Table 6).

There are a selection of histologic features that are associated with a more aggressive biologic behavior: capsular invasion, vascular invasion (separate from vena cava invasion), tumor necrosis, increased mitotic activity (>15/20 HPF), and the presence of atypical mitotic figures.[2,15,43] Whereas necrosis and >15 mitotic figures per 20 HPF independently suggest a worse clinical outcome based on multivariate analysis, none of these features can be used solely as a predictor of malignancy: necrosis was present in 59% of the cases in group B, patients who had a good clinical outcome. Therefore, 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 (Table 8) must be taken into consideration before a diagnosis of ACC can be made in pediatric patients.

In the purest sense possible, recurrent or metastatic disease (lymph node and/or other organ site) was taken to be indicative of a malignant primary tumor. Keep in mind that there was significant overlap in histologic features between group B and C tumors. With this endpoint in mind, we attempted to separate the clinically benign tumors (n = 60) from those that were clinically malignant (n = 23) based on the number of criteria present (Table 9). It is important in a grading 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. All patients who had developed documented metastatic disease in this clinical study had anywhere from 1 to 9 histologic criteria present (Tables 8 and 9), whereas the patients with a good clinical outcome had from 0 to 7 histologic criteria present. Therefore, there is no clean breakpoint above or below which patients can be accurately classified. However, it is with great interest that the two patients in the clinically malignant category who each only had one of the criteria (necrosis and atypical mitotic figures, respectively) are both alive without evidence of disease at the last follow-up. Therefore, although they developed recurrent disease in the bed of the tumor at 4 and 14 months, respectively, they did not die from their disease. Consequently, in an effort not to overcall tumors that behave in a benign clinical fashion nor undercall tumors that result in patient death, we propose a three-part separation: up to two criteria, benign long term clinical outcome; three criteria: indeterminate for malignancy (intermediate, atypical, uncertain malignant potential); and four or more criteria, portends a poor clinical outcome (Table 9). These breakpoints accurately classify 78% of all cases that behave in a clinically malignant fashion; 58% of all cases that behave in a clinically benign fashion; and 22% of patients are placed in an indeterminate category, of which 4% behave in a clinically malignant fashion.

This means that of the entire group of 83 patients in this study, 37 would now be placed in the good clinical outcome category (two or fewer criteria; no patient in this group died from their disease); 18 patients would be placed in the intermediate category (three criteria; 17% [three patients] went on to behave in a clinically malignant fashion); and 28 patients would be placed in the poor clinical outcome category (four or more criteria; 64% [18 patients] went on to behave in a clinically malignant fashion). Therefore, while no documented metastases were identified in 10 patients in the poor patient outcome category, the tumors had similar morphologic features of malignancy to those in the neoplasms that metastasized. Thus, although this separation will hopefully provide a valuable adjunct in the prospective management of patients, a specific number of pathologic criteria in no way guarantees the development of metastatic disease nor dictates that the patient will die from their disease. Neither does a specific number guarantee a benign outcome.

Although this proposed prospective pathology separation of adrenal cortical neoplasms in pediatric patients has statistically significant utility in this clinical series, it

TABLE 8. *Proposed criteria for malignancy of adrenal cortical neoplasms in pediatric patients*

Macroscopic and microscopic criteria for malignancy of adrenal cortical neoplasms in pediatric patients
Tumor weight of >400 g
Tumor size >10.5 cm
Extension into periadrenal soft tissues and/or adjacent organs
Invasion into vena cava
Venous invasion
Capsular invasion
Presence of tumor necrosis
>15 mitoses per 20 HPF
Presence of atypical mitotic figures

HPF, high-power field (400×).

TABLE 9. *Separating clinically benign from clinically malignant pediatric adrenal cortical neoplasms*

Cumulative no. of criteria present*	No. of clinically benign cases	No. of clinically malignant cases
0	10	0
1	17	2
2	8	0
3	15	3
4	5	3
5	2	4
6	2	5
7	1	5
8	0	0
9	0	1

* Criteria as listed in Table 8.

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, which had aggressive clinical behavior (although the patients are alive without disease now) initially with a score of 1, or the patient with a score of 7, who has enjoyed a benign clinical course. We propose that this system of separation be applied to a larger number of cases in general clinical practice to determine its clinical utility and validity because any one of the features documented in malignant cases (including necrosis, increased mitotic figures, and atypical mitotic figures) have been described in tumors that did not go on to recur or develop metastatic disease.

We did not find immunohistochemical evaluation to be prognostically valuable, although the strong inhibin immunoreactivity may be of value in differentiating adrenal cortical neoplasms from other neoplasms in the differential diagnostic workup.[12,25,26] In general, adrenal cortical neoplasms show variable, typically weak, expression for cytokeratin intermediate filaments,[7,32,33,35,41] with CK5/CK6 expressed most frequently in this series.

Based on this clinical study, we conclude that adrenal cortical tumors in children are both clinically and histologically different from those occurring in adults, even though there is overlap.[3,8,11,15] Our data indicate that although adrenal cortical neoplasms in childhood may not be as uniformly fatal as they are in adults, even with histologic evidence of malignancy,[3,34] when they are clinically malignant, they are relatively aggressive, resulting in death within a relatively short period of time (<2 years). However, 5- and 10-year disease-free survival rates of 63.9% and 59.0%, respectively, suggest that once a patient lives beyond 5 years, prognosis appears to be quite good.[8,24,27,30,31,36] The role for radiation and/or chemotherapy remains controversial[21,24,36] and requires additional investigation once a more accurate determination of pathologic criteria have been applied. No one pathologic feature can be used to accurately and independently predict patient outcome. However, we have identified several factors, which when identified in aggregate, appear to accurately predict a more aggressive biologic behavior (Table 8). As more features are seen, a trend toward malignant behavior is definitely identified. By using a cutoff of four or more features, more aggressive clinical management and follow-up may yield an improvement in the overall patient outcome. It is axiomatic, but perhaps needs emphasis, that the intermediate/indeterminate group of patients will need to have clinical follow-up similar to those that are frank carcinomas, at least until a 5-year disease-free survival period is achieved to exclude a more aggressive biologic behavior. Application of these criteria to adrenal cortical neoplasms in children in general practice is encouraged to further substantiate these initial findings. □

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