Adrenocortical Carcinoma: Prognostic Indices Based on Clinical and Immunohistochemical Markers

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ABSTRACT

Adrenocortical carcinoma is a rare condition with an unpredictable prognosis as a rule. The authors retrospectively analyzed the clinical outcome of 46 patients (31 F, 15 M) during 16 years building up a numerical index for the prognosis, based on clinical and immunohistochemical data. Four indices were analyzed:

$$J1 = \frac{Y + 2L + 4H}{T}; \quad J2 = (J1)\sqrt{\frac{W}{200}};$$

$$J3 = \frac{O + Y + 2L + 4H}{T}; \quad J4 = (J3)\sqrt{\frac{W}{200}}.$$

Y = 1 when chronological age (CA) >33 mo, Y = 0 when CA ≤33 mo; L = 1 for right sided tumor and L = 0 for left sided tumor; H = 1 in presence of hypertension and H = 0 for normal blood pressure; T = length of disease in months; W = weight of tumor (g); O = 1 in the absence of p53 protein and O = 0 in the presence of p53. The chance of bad prognosis was observed when age is >33 mo, tumor is on the right side, systemic hypertension is present, tumor weight >250 g, in the absence of p53, *J1*, *J2*, *J3* >0.4 (p <0.001) and *J4* >0.5 (p <0.01). Clinical data and the mathematical model enabled us to establish probabilities of good prognosis in 78-96% and bad prognosis in 63-83%.

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INTRODUCTION

Adrenocortical carcinoma (AC) is rare in children and adolescents, having a variable and potentially fatal course characterized by elevated production of adrenal hormones. In the last 16 years we have registered 46 cases, while in other countries the numbers are not so striking[1-6].

The diagnosis is usually made on clinical grounds, since the majority of AC in children express endocrine manifestations, such as Cushing's syndrome, virilization, precocious puberty and systemic hypertension. Surgical removal of a localized tumor, in cases without metastatic disease, is the most effective form of therapy but does not determine the prognosis. Neither histological studies, according to Medeiros and Weiss[12], nor the quantification of the DNA content and tumor size, have proven to be a sufficient basis for establishing the prognosis of the AC[5,7-13]. With this aim, immunohistochemical and molecular studies have been suggested as the most appropriate tools to study the behavior of this type of tumor[14-17].

Immunohistochemical studies were undertaken with the objective of determining the biological behavior of the neoplastic cells, and in particular the proliferating cell nuclear antigen (PCNA), which could be a good marker of cellular multiplication giving a prognostic index[18]. The p53 gene has been the subject of various studies and it has been named the 'guardian of the genome', protecting DNA either by blocking the cell cycle or by inducing

apoptosis in disordered cells[19-22]. In special situations, when a mutation occurs, the activity of the gene reverts to such an extent that its modified protein promotes cell transformation and tumorigenesis[17-24].

Based on the difficulty of the prognosis for AC in pediatric patients, we decided to use mathematical formulas to provide a numerical index derived from clinical and immunohistochemical data regarding to p53 protein, with the objective of determining a more reliable prognosis and hence a more appropriate therapeutic strategy[25-27].

PATIENTS AND METHODS

Forty-six children and adolescents (31 girls and 15 boys) were enrolled in this retrospective study. The main complaints were the presence of virilization or precocious puberty. Twenty-eight were alive after 20 months to 16 years of follow up, and 18 had a malignant outcome: seven metastasized widely and 11 died. The characteristics of the patients are summarized in Table 1. The histological study of 33 patients was based on the criteria of Medeiros and Weiss[12], which take into account nine items: 1. high nuclear grade; 2. presence of over five mitoses per 50 high-power fields; 3. atypical mitotic figures; 4. 75% or more of the cells with eosinophilic cytoplasms; 5. 33% or more of the tumoral architecture being diffuse; 6. the presence of necrosis; 7. tumoral invasion of the smooth musculature of the venous vascular wall; 8. sinusoidal invasion; and 9. capsular invasion. The presence of three or more of the above criteria suggests malignant tumoral behavior.

For the immunohistochemical study of the altered p53 protein, we used sections of paraffin-embedded blocks and DO-1 monoclonal antibodies (Santa Cruz Technology, USA) which are specific for paraffin-embedded samples. We considered the presence of specific staining either in the nucleus or in the cytoplasm as positive.

Data were statistically analyzed with respect to the following: age of the patient (Y); laterality of the tumor (L); presence of systemic hypertension (H); tumor weight (W); immunohistochemical aspects of the p53 protein (O); and delay from the onset of symptoms to the time of diagnosis (T).

We created four indices, *J*1, *J*2, *J*3 and *J*4 (see below), with the objective of establishing a single variable which contains all information, such as the patient's age, laterality of the tumor, systemic hypertension, duration of disease, tumor size and immunohistochemical properties of the p53 protein. The variables placed in the numerator with a positive sign and multiplied by a given value are directly related to the development of the disease. The duration of disease variable is inversely related and placed as the denominator. To ensure an easily compared final value the square root divided by 200 is justified by the high numerical value of the weight (g). To demonstrate the close correlation between these indices and the development of the disease, we applied a Wilcoxon test[24] to analyze the 'benign course' in opposition to the 'malignant course' groups. To evaluate the results, we categorized the index and performed a χ^2 test. The median for *J*1, *J*2, and *J*3 is 0.4, while for *J*4 it is 0.5. We estimated the probabilities of the prognosis according to these indices.

The *J*1 index took into consideration the duration of the disease, T (months); age of the patient (when over 33 months Y = 1 and when less than or equal to 33 months Y = 0); site of the tumor, L = 1 (right) and L = 0 (left); and H = 1 or H = 0 if systemic hypertension is present or absent, respectively. With the 45 patients (excluding those for whom the tumor size was not known), we reached *J*2 where W is the tumor weight in grams. For the 33 patients who had their p53 gene analyzed (O), we also defined two indices: *J*3 for unknown tumor weight, and *J*4 (n = 32) for known tumor weight. If immunohistochemical tests are negative, O = 1; if positive, O = 0.

$$J1 = \frac{Y + 2L + 4H}{T} \; ; \quad J2 = J1\sqrt{\frac{W}{200}} \; ;$$

$$J3 = \frac{O + Y + 2L + 4H}{T} \; ; \quad J4 = J3\sqrt{\frac{W}{200}} \; .$$

RESULTS

Although there was a clear predominance of females (n = 31, 67.4%) compared to males (n =

TABLE 1: Characteristics of the adrenocortical carcinoma in 46 patients

Patient #	Sex	Y	L	H	T	W	O	Course
1	F	24 / 0	0	1	4	160	0	0
2	F	50 / 1	1	0	4	250	1	1
3	F	33 / 0	0	0	9	330	1	0
4	F	9 / 0	0	1	2	70	1	1
5	F	24 / 0	0	0	12	85	1	0
6	F	13 / 0	0	0	3	110	0	0
7	F	77 / 1	1	1	6	430	1	1
8	F	120/ 1	1	1	7	1,295	1	1
9	F	66 / 1	1	0	6	1,430	0	1
10	F	22 / 0	1	0	15		0	0
11	F	66 / 1	1	0	2	750	0	1
12	F	39 / 1	1	1	18	770	1	1
13	F	122/ 1	0	1	6	1,100	0	1
14	F	22 / 0	0	1	9	65	0	0
15	F	22 / 0	1	1	3	30	0	1
16	F	4 / 0	0	1	2	80	0	0
17	F	14 / 0	0	0	3	30	1	0
18	F	59 / 1	0	0	30	19	1	0
19	F	23 / 0	0	1	5	50	0	0
20	F	57 / 1	0	0	30	50	1	0
21	F	31 / 0	0	0	24	55	0	0
22	F	15 / 0	0	1	7	50	1	0
23	F	30 / 0	0	0	12	15	0	0
24	M	22 / 0	0	0	12	24	0	0
25	M	38 / 1	1	0	12	1,530	1	1
26	M	32 / 0	0	1	18	15	0	0
27	M	68 / 1	1	1	20	350	0	1
28	M	33 / 0	1	0	21	45	0	0
29	M	100 / 1	0	0	1	350	1	1
30	M	19 / 0	0	1	12	120	0	0
31	M	47 / 1	0	1	1	800	1	1
32	M	120 / 1	0	1	1	700	1	1
33	M	36 / 1	0	0	5	39	0	0
34	F	36 / 1	1	0	12	15		0
35	F	147 / 1	0	1	12	600		1
36	F	66 / 1	1	1	22	360		1
37	F	41 / 1	0	0	5	382		0
38	F	69 / 1	0	0	5	5		0
39	F	17 / 0	1	0	6	630		0
40	F	148 / 1	0	1	2	1,380		1
41	F	58 / 1	0	0	19	10		0
42	M	14 / 0	0	1	8	10		0
43	M	67 / 1	0	1	1	1,000		1
44	M	4 / 0	1	1	2	150		0
45	M	106 / 1	1	0	12	15		0
46	M	39 / 1	1	1	7	80		0

Y: age (months). Age >33 months Y = 1; <33 months Y = 0. L: side of tumor: right L = 1; left L = 0.
H: systemic hypertension: present H = 1; absent H = 0. T: duration of disease in months. W: tumor weight in grams.
O: p53 protein immunohistochemistry: negative O = 1; positive O = 0. Course: bad = 1; good = 0.

15, 32.6%), there was no correlation between the sex of the patients and a good or bad outcome ($p = 0.60$). The same occurred with the criteria of Medeiros and Weiss[12] ($p = 0.37$).

We observed a higher probability of bad prognosis among patients aged over 33 months ($p < 0.01$), duration of disease less than or equal to 7 months ($p < 0.05$), tumor on the right side ($p < 0.05$), presence of systemic hypertension ($p < 0.05$), tumor weight greater than 250 g ($p < 0.001$) and immunohistochemical test negative for p53 protein ($p = 0.06$). *J*1, *J*2 and *J*3 with values greater than 0.4 ($p < 0.01$) and *J*4 greater than 0.5 ($p < 0.01$) indicate bad prognosis (Table 2).

The calculation of the associated probability with the indices *J*1, *J*2, *J*3 and *J*4 showed a possibility of a good outcome in 78-96% of the patients and a bad one in 63-83% (Table 3).

DISCUSSION

All of our patients had functioning tumors that caused virilization in 98% of the girls and precocious puberty in 93% of the boys. Bad prognosis was recorded in 18 patients (39.1%). Literature data indicate that relapse generally occurs within the first 18 months, and after this time there is a very good chance of cure[9,29-32], similar to what has been observed in our own patients.

Different from reports in the literature, our study shows that girls are more affected than boys (2:1), probably because it is easier for families to notice the virilization of a girl as opposed to the signs of isosexual puberty in a boy. Despite the predominance of females with AC, there was no difference between the sexes with regard to bad prognosis.

Another relevant fact in our study is the discrepancy in the histopathological findings and the follow up of the patients, confirming recent observations in the literature regarding the low prognostic value of this method, as well as PCNA analysis[33,34]. According to some authors, the nuclear content of the DNA may have a prognostic value, but there is a lot of controversy in recent studies regarding the use of this method to determine the malignant potential[11,35,36].

A positive fact observed in this study is the higher incidence of bad outcome in patients with a duration of disease less than or equal to 7 months, demonstrating that the greater the velocity of clinical events, the more aggressive the tumor. On the other hand, the older the child, the worse the prognosis. This finding calls attention to the possibility of late diagnosis in our patients. Another important observation was the presence of systemic hypertension as a marker of bad prognosis, even in children who did not present signs of Cushing's syndrome, demonstrating a more prominent mineralocorticoid activity, characterizing a mixed hormonal profile.

In 1987, Brennan[37] observed that tumors located on the right side had more aggressive behavior, in accordance with our results. A possible explanation for this finding could be that the right-sided tumor is very close to the inferior vena cava and its venous drainage is made directly into it, facilitating tumoral dissemination.

Tumor size is an important prognostic marker, but it should be highlighted that some patients with a tumoral weight greater than 250 g had a benign course (patients # 3, 37 and 39), while only three patients (patients # 2, 4 and 15) with a tumoral weight less than or equal to 250 g presented a bad outcome. Several authors have also reported the importance of the weight or volume of the tumor as a prognostic marker[5,34,35,37,38].

Study of the p53 protein, using a specific monoclonal antibody immunohistochemical method, allowed a retrospective study of paraffin-embedded tumoral material, giving a positive reactivity of approximately 54% when compared with the literature (between 19% and 27%)[24,35,39]. However, the most curious finding was the negative reactivity seen in cases with bad outcome, and positive reactivity in greater numbers among the cases with good prognosis. Perhaps the poor outcome in the negative cases is associated with very important gene alterations and production of extremely modified protein whose altered immunogenicity precludes its detection, as demonstrated by Reincke *et al.*[39] in AC in adults via gene amplification.

The statistical analysis and mathematical model primarily enabled a prognostic evaluation based only on clinical data, as demonstrated by the *J*1 index, whose variables are directly related to the patient's age, laterality of the tumor, presence of

TABLE 2

Follow up of adrenal cortex carcinoma

	Bad	Good	Total	p
Age >33 mo	16	9	25	
Age ≤33 mo	2	19	21	<0.0009
Tumor side R	10	7	17	
Tumor side L	8	21	29	<0.036
Hypertension present	13	10	23	
Hypertension absent	5	18	23	<0.01
Length disease ≤7 mo	14	12	26	
Length disease >7mo	4	16	20	<0.01
Tumor weight ≤250 g	3	24	27	
Tumor weight >250 g	15	3	18	0.000001
p53 negative	9	6	15	
p53 positive	5	13	18	0.06
***J*1 ≤0.4**	3	20	23	
***J*1 >0.4**	15	8	23	0.0002
***J*2 ≤0.4**	0	22	22	
***J*2 >0.4**	18	5	23	0.0000001
***J*3 ≤0.4**	3	13	16	
***J*3 >0.4**	11	6	17	0.007
***J*4 ≤0.5**	0	16	16	
***J*4 >0.5**	14	2	16	0.000001

TABLE 3

Associated probabilities

Pr{B| *J*1 >0.4} = 64%	Pr{B| *J*2 >0.4} = 76%
Pr{G| *J*1 ≤0.4} = 84%	Pr{G| *J*2 ≤0.4} = 96%
Pr{B| *J*3 >0.4} = 63%	Pr{B| *J*4 >0.5} = 83%
Pr{G| *J*3 ≤0.4} = 78%	Pr{G| *J*4 ≤0.5} = 94%

Pr = probability; B = bad; G = good; *J*1-4: indices.

systemic hypertension, and duration of disease. This index demonstrated a probability associated with a good outcome in 84% and a bad one in 64% of cases. When we associated the negative reactivity of the p53 protein to this index, the probability of a good outcome was 78% and a bad one 63%. Adding the weight of the tumor to the *J2* and *J4* indices, we obtained the probabilities for a good outcome in 96% and 94%, and a poor outcome in 76% and 83%, respectively.

This study led us to conclude that the histopathological information does not correlate with the probable course and outcome of the disease. On the other hand, clinical data and tumoral weight are important as prognostic tools. The proposal of a simple mathematical model aims to offer a useful tool to deal with the prognosis of patients with AC.

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