Texture Image Analysis in Differentiating Malignant from Benign Adrenal Cortical Tumors in Children and Adults

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Abstract. *Objective: To investigate the possible role of chromatin texture parameters, nuclear morphology, DNA ploidy and clinical functional status in discriminating benign from malignant adrenocortical tumors (ACT). Patients and Methods: Forty-eight cases of clinically benign (n=40) and clinically malignant (n=8) ACT with a minimum of 5-years' follow-up were evaluated for chromatin texture parameters (run length, standard deviation, configurable run length, valley, slope, peak and other 21 Markovian features that describe the distribution of the chromatin in the nucleus), nuclear morphology (nuclear area, nuclear perimeter, nuclear maximum and minumum diameter, nuclear shape), and DNA ploidy. Nuclear parameters were evaluated in Feulgen-stained 5 μm paraffin-sections analyzed using a CAS 200 image analyzer. Results: Since ACTs present different biological features in children and adults, patients were divided into two groups: children (≤ 15 years) and adults (>15 years). In the group of children DNA ploidy presented a marginal significance (p=0.05) in discriminating ACTs. None of the parameters discriminated between malignant and benign ACT in the adult group. Conclusion: ACTs are uncommon and definitive predictive criteria for malignancy remain uncertain, particularly in children. Our data point to DNA content evaluated by image analysis as a new candidate tool for this challenging task. Texture image analysis did not help to differentiate malignant from benign adrenal cortical tumors in children and adults.*

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Key Words: Adrenocortical tumors, adrenal, image analysis, chromatin texture, nuclear morphology, DNA ploidy, children, adults.

The distinction between benign and malignant adrenocortical tumors (ACTs) is often difficult, particularly in children (1-6)). Although in adult Weiss' histological score system has been useful for such distinction (7-10), borderline cases are still encountered. These indeterminate cases are often small tumors with several worrisome histological features – a category of tumor that is becoming more frequently encountered with the increasing use of noninvasive imaging techniques such as computerized tomography and magnetic resonance imaging.

Quantification in histopathology has the goal of bringing objectivity to diagnostic assessment and of improving the diagnostic and prognostic capabilities. The distributive pattern of chromatin in the cell nuclei is a very sensitive indicator of changes in cell function and has been used as a major criterion for diagnosis of malignancy throughout history, based on visual estimates by conventional optical microscopy. Chromatin texture by computer-assisted image analysis has shown distinctive patterns in preneoplastic and neoplastic lesions of the prostate (10-13), breast (14, 15) and cervix (16, 17). Only two previous studies have evaluated chromatin texture parameters in ACT to highlight the possible role of these kind of parameters in helping to discriminate benign from malignant ACT (18, 19).

Table I. *Group of patients.*

	Tumor biological behavior		Gender		Age (years)	
	Benign	Malignant	Male	Female	Mean	Range
Children	15	3	4	14	3.7	0.6-15
Adults	25	5	4	26	33.4	19-53

Materials and Methods

Patients. Forty-eight cases of clinically benign (n=40) and clinically malignant (n=8) ACT were included in the study. Patients were submitted to surgical resection of ACT at the Clinical Hospital in the period between 1979 and 1994, and had a minimum of 5-years' follow-up. All pertinent clinical information was obtained from clinical records. Paraffin blocks and pathological reports were retrieved from the files of the Surgical Pathology Division of the Clinical Hospital. Metastasis or disease-recurrence was considered as evidence of malignancy. Patients were divided into two groups: children (≤15 years) and adults (>15 years) (Table I)

Nuclear parameters. Five μm sections representative of the tumor in each case were submitted to Feulgen staining and image analysis using the CAS 200 system (Becton and Dickinson). Briefly, the slides were placed in 10% neutral formaldehyde for 30 minutes and air-dried. Afterwards, hydrolysis was performed with 5 mM HCl for 60 minutes at room temperature. Subsequently, the Thyonin-Feulgen (Becton and Dickinson) stain was applied, mounted with Entellan (Merck, Germany) and slides were coverslipped.

Nuclear *DNA* content analysis was carried out using the CAS 200 system (Becton and Dickinson), provided with quantitative measurement 3.0 (v. 8.1) (Becton & Dickinson, USA). Optical density was evaluated for 150 to 200 cells per case using x400 total magnification. Overlapping images and multinucleated cells were excluded from the analysis. The system was calibrated with rat hepatocytes (slide control – Becton and Dickinson) as an external control. Normal adrenal cortical cells or stromal cells present in the sample were used as internal diploid reference. According to DNA histograms, cases were classified as DNA diploid if the DNA index was in the range of 0.90-1.10; DNA tetraploid, in the range of 1.80-2.20 and DNA aneuploid in any other situation.

Chromatin texture parameters were evaluated using Cell View™ (Version 1.0) and Cell Sheet™ (Version 2.0) software (Bacus Laboratories Inc., Elmhurst, IL, USA); nuclear images were extracted from Feulgen-stained 5 μm paraffin-sections as intermediate level maintenance (ILM) files. These nuclei were analyzed by CAS 200 image analyzer by two of us (RM and AS); the following features were evaluated for each measured nucleus: Nuclear area, nuclear perimeter, nuclear maximum diameter, nuclear minimum diameter and nuclear shape for nuclear morphology; Run length, standard deviation, configurable run length, valley, slope, peak and 21 other Markovian features that describe the distribution of the chromatin in the nucleus. These features derive from the transition probability matrix that is obtained reducing the optical density histogram (256 gray levels) of the nucleus into eight gray levels for the chromatin texture analysis.

Table II. *Area and chromatin texture parameters and biological behavior of tumors in children.*

Parameter	Biological behavior	Mean rank	Sum of ranks	*P*-value*
Area	Benign	9.93	149	0.49
	Malignant	7.33	22	
Run length	Benign	10.33	155	0.16
	Malignant	5.33	16	
Cfg. run length	Benign	10.0	150	0.42
	Malignant	7.0	21	
Valley	Benign	10.33	155	0.16
	Malignant	5.33	16	
Slope	Benign	10.4	156	0.13
	Malignant	5.0	15	
Peak	Benign	9.13	137	0.57
	Malignant	11.33	34	

Cfg., Configurable. *Mann-Whitney test; benign=15; malignant=3.

Briefly, the meaning of some of these parameters is described below: Run length: count of the pixels within the nucleus whose gray level values differ from those of their left and right neighbors; Configurable run length: similar to run length with the possibility of configuring the sample size and the difference threshold of the pixel and the projection of the count; Valley, slope, peak: count of the pixels within the nucleus that have: two neighboring pixels with higher gray level values (valley), two neighboring pixels with a higher and a lower gray level value (slope), two neighboring pixels with lower gray level values (peak); standard deviation: standard deviation of optical density of every pixel that composed the nucleus.

Statistical analysis. Mean values of chromatin texture features and nuclear area were compared in clinically benign and malignant tumors using the Mann-Whitney *U*-test. Functional status and DNA ploidy category were compared in benign and malignant tumors using Fisher's exact test. A difference was considered significant when the type I error was less than 0.05.

Results

The group of patients comprised 18 children and 30 adults. The gender distribution and mean age are shown in Table I. Results for area and chromatin texture analysis and biological behavior of the tumor in children are presented in Table II; the non-parametric Mann-Whitney test did not show any significant results. A similar analysis for adults is presented in Table III but again no significant result was observed. Table IV shows the evaluation of DNA ploidy as related to the biological behavior of the tumors in children and adults. Fischer's exact test showed marginal significance (p=0.05) in the children's group, but not in that of the adults.

Among all parameters, in the group of children only DNA ploidy presented a marginal statistical significance (p=0.05). None of the parameters studied discriminated between benign and malignant ACT in the adult group.

Table III. *Area and chromatin texture parameters and biological behavior of tumors in adults.*

Parameters	Biological behavior	Mean	*P*-value*
Area	Benign	14.88	0.41
	Malignant	18.60	
Run length	Benign	15.04	0.55
	Malignant	17.80	
Cfg. run length	Benign	15.04	0.55
	Malignant	17.80	
Valley	Benign	14.72	0.30
	Malignant	19.40	
Slope	Benign	14.64	0.25
	Malignant	19.80	
Peak	Benign	16.28	0.30
	Malignant	11.60	

Cfg., Configurable. *Mann-Whitney test; benign=25; malignant=5

Table IV. *DNA ploidy and biological behavior in tumors from children and adults.*

DNA ploidy	Benign	Malignant	Total	*P*-value*
Children				
Diploid	33.33%	66.7%	100%	
Aneuploid + tetraploid	100%		100%	**0.05**
Total	81.8%	18.2%	100%	
Adults				
Diploid	83.3%	16.7%	100%	
Aneuploi + tetraploid	80.0%	20%	100%	0.61
Total	81.5%	18.5%	100%	

*Fischer's exact test.

Discussion

ACTs are uncommon. In the USA, only about 25 new cases occur each year and constitute only about 0.2% of all pediatric malignancies. However, the incidence of ACT varies across geographical regions and is remarkably high in southern Brazil, particularly in children, where it is 10-15 times higher than that of pediatric ACT worldwide (20, 21).

The most important point to be defined in a patient with an ACT is the differential diagnosis between adenoma and carcinoma. There is no single histological criterion which can reasonably differentiate adrenocortical carcinoma from adenoma such as capsular or vascular invasion in thyroid follicular carcinoma. Among various criteria used, the criteria proposed by Weiss (7, 8), which evaluate nine histological features of the tumor most frequently associated with poor clinical outcome of the patients, have been most widely employed due to the straightforwardness of the criteria and easy applicability. In previous reports (2, 3, 5, 6, 9), the Weiss score system is reliable and easily used in routine practice and is a good predictor of clinical behavior in adults with ACT. However, this is not the case in children, where most cases classified as adrenocortical carcinoma by these criteria will present a benign clinical course. Recently, molecular and cellular features of ACT have been studied (22), but the relatively low frequency of this disease, the lack of a well established pre-malignant condition in human adrenal cortex and the marked heterogeneity in morphology and biological function have contributed to the lack of definitive conclusions helpful to this practical and important issue.

Cellular features such as chromatin texture, nuclear morphology and DNA content analysis have been studied by different groups in ACT in both children and adults. Scarpelli *et al*. (18, 19) described different findings regarding nuclear size, chromatin pattern and DNA content in 10 adrenocortical adenomas and 5 adrenocortical carcinomas, analyzed by image analysis. As far as we know, no other data regarding chromatin texture in ACT have been published.

Regarding DNA ploidy, Zerbini *et al*. (2) and Bugg *et al*. (23) did not find a statistically significant difference in DNA ploidy evaluated by image analysis in a series exclusively of pediatric patients. Other series, including children and adults together, demonstrated positive (24, 25) or negative (26) correlation between ploidy and malignancy defined by pathology or clinical behavior. Controversial results have been attributed to four important considerations: first, ACTs in children differ significantly from similar neoplasms in adults and must be evaluated separately; second, different methods, including image analysis and flow cytometry, used especially for DNA content analysis may have technical differences to be considered before comparison of results can be made; third, because of the rarity of these tumors, the number of cases is generally small, and lastly, ideally, every study should have as an endpoint the clinical behavior, defined by the occurrence of recurrent or metastatic disease, especially in the pediatric age group. Interestingly, in this series, DNA non-diploid tumors were associated with benign neoplasms in children (see Table IV), as usually occurs with other types of pediatric cancer such as neuroblastoma (27) and acute lymphoblastic leukemia (28).

These findings did not show a statistically significant correlation of the chromatin texture parameters, but did demonstrate a marginally significant correlation of DNA ploidy with clinical behavior in children, suggesting that this feature should be added to morphological parameters recently defined in pediatric ACT (4) for validation in larger series collected by the International Pediatric Adrenocortical Tumor Registry (29).

Acknowledgements

The authors are in debt to Eduardo Garcia for his technical assistance in histochemical procedures.

References

1 Ribeiro RC, Sandrini Neto RS, Schell MJ, Lacerda L, Sambaio GA and Cat I: Adrenocortical carcinoma in children: a study of 40 cases. J Clin Oncol *8*: 67-74, 1990.

2 Zerbini C, Kozakewicn HPW, Weinberg DS, Mundt DJ, Edwards JA III and Lack EE: Adrenocortical neoplasms in childhood and adolescence: analysis of prognostic factors including DNA content. Endocr Pathol *3*: 116-128, 1992.

3 Mendonça BB, Lucon AM, Menezes CA, Saldanha LB, Latronico AC, Zerbini C, Madureira G, Domenice S, Albergaria MA, Camargo MH, Madureira G, Domenice S, Pereira MAA, Camargo MHA, Halpern A, Liberman B, Arnhold IJP, Bloise W, Andriolo A, Nicolau W, Silva, FAQ, Wroclaski E and Wajchenberg BL: Clinical, hormonal and pathological findings in a comparative study of adrenocortical neoplasms in childhood and adulthood. J Urol *154*: 2004-2009, 1995.

4 Wieneke JA, Thompson LD and Heffess C: Adrenal cortical neoplasms in the pediatric population: a clinicopathologic and immunophenotypic analysis of 83 patients. Am J Surg Pathol *27*: 867- 81, 2003.

5 Sredni ST, Alves VA, Latorre Mdo R and Zerbini MC: Adrenocortical tumours in children and adults: a study of pathological and proliferation features. Pathology *35*: 130-135, 2003.

6 Dehner LP: Pediatric adrenocortical neoplasms: on the road to some clarity. Am J Surg Pathol *27*: 1005-1007, 2003.

7 Weiss LM: Comparative histologic study of 43 metastasizing and nonmetastasizing adrenocortical tumors. Am J Surg Pathol *8*: 163-9, 1984.

8 Medeiros LJ and Weiss LM: New developments in the pathologic diagnosis of adrenal cortical neoplasms. A review. Am J Clin Pathol *97*: 73-83, 1992.

9 Aubert S, Wacrenier A, Leroy X, Devos P, Carnaille B, Proye C, Wemeau JL, Lecomte-Houcke M and Leteurtre E: Weiss system revisited: a clinicopathologic and immunohistochemical study of 49 adrenocortical tumors. Am J Surg Pathol *26*: 1612-1619, 2002.

10 Lau SK and Weiss LM: Adrenocortical neoplasms. Pathol Case Rev *10*: 219-227, 2005.

11 Christen R, Xiao J, Minimo C, Gibbons G, Fitzpatrick BT, Galera-Davidson H, Bartels PH and Bibbo M: Chromatin texture features in hematoxylin and eosin-stained prostate tissue. Anal Quant Cytol Histol *15*: 383-388, 1993.

12 Bostwick DG: Practical clinical application of predictive factors in prostate cancer. A review with an emphasis on quantitative methods in tissue specimens. Anal Quant Cytol Histol *20*: 323- 342, 1998.

13 Bartels PH, da Silva VD, Montironi R, Hamilton PW, Thompson D, Vaught L and Bartels HG: Chromatin texture signatures in nuclei from prostate lesions. Anal Quant Cytol Histol *20*: 407- 416, 1998.

14 Bostwick DG, Grignon DJ, Hammond ME, Amin MB, Cohen M, Crawford D, Gospadarowicz M, Kaplan RS, Miller DS, Montironi R, Pajak TF, Pollack A, Srigley JR and Yarbro JW: Prognostic factors in prostate cancer. College of American Pathologists Consensus Statement 1999. Arch Pathol Lab Med *124*: 995-1000, 2000.

15 Poulin N, Frost A, Carraro A, Mommers E, Guillaud M, Van Diest PJ, Grizzle W and Beenken S: Risk biomarker assessment for breast cancer progression: replication precision of nuclear morphometry. Anal Cell Pathol *25*: 129-138, 2003.

16 Mariuzzi L, Mombello A, Rucco V, Morelli L, Zamo A, Thompson D, Vaught L, Bartels HG, Mariuzzi G and Bartels PH: Quantitative study of ductal breast cancer progression: signatures of nuclei in proliferating breast lesions and in situ cancer. Adv Clin Path *4*: 87-97, 2000.

17 Drezek R, Guillaud M, Collier T, Boiko I, Malpica A, Macaulay C, Follen M and Richards-Kortum R: Light scattering from cervical cells throughout neoplastic progression: influence of nuclear morphology, DNA content, and chromatin texture. J Biomed Opt *8*: 7-16, 2003.

18 Nemec E, Van de Putte S, Van Pachterbeke C, Vokaer R, Budel V, Deprez C, Kiss R and Decaestecker C: Ploidy and chromatin pattern analysis as an aid for cervical smear diagnosis. Histol Histopathol *17*: 403-409, 2002.

19 Scarpelli M, Baccarini MG, Colanzi P, Arnaldi G, Montironi R, Thompson D and Bartels PH: Chromatin texture analysis of cortical adrenal gland adenomas, including incidentalomas, and adjacent normal-appearing cortical tissue. Anal Quant Cytol Histol *22*: 235-243, 2000.

20 Scarpelli M, Montironi R, Mazzucchelli R, Thompson D and Bartels PH: Distinguishing cortical adrenal gland adenomas from carcinomas by their quantitative nuclear features. Anal Quant Cytol Histol *2*: 131-138, 1999.

21 Ribeiro RC and Figueiredo B: Childhood adrenocortical tumours. Eur J Cancer *40*: 1117-1126, 2004.

22 Almeida MQ and Latronico AC: The molecular pathogenesis of childhood adrenocortical tumors. Horm Metab Res *39*: 461-466, 2007.

23 Bugg MF, Ribeiro RC, Roberson PK, Lloyd RV, Sandrini R, Silva JB, Epelman S, Shapiro DN and Parham DM: Correlation of pathologic features with clinical outcome in pediatric adrenocortical neoplasia. A study of a Brazilian population. Brazilian Group for Treatment of Childhood Adrenocortical Tumors. Am J Clin Pathol *101*: 557-558, 1994.

24 Pignatelli D, Leitao D, Maia M and Schmidt F: DNA quantification and ploidy patterns in human adrenocortical neoplasms. Endocr Res *24*: 869-874, 1998.

25 Suzuki T, Sasano H, Nisikawa T, Rhame J, Wilkinson DS and Nagura H: Discerning malignancy in human adrenocortical neoplasms: utility of DNA flow cytometry and immunohistochemistry. Mod Pathol *5*: 224-231, 1992.

26 Cibas ES, Medeiros LJ, Weinberg DS, Gelb AB and Weiss LM: Cellular DNA profiles of benign and malignant adrenocortical tumors. Am J Surg Pathol *14*: 948-955, 1990.

27 Ladenstein R, Ambros IM, Potschger U, Amann G, Urban C, Fink FM, Schmitt K, Jones R, Slociak M, Schilling F, Ritter J, Berthold F, Gadner H and Ambros PF: Prognostic significance of DNA di-tetraploidy in neuroblastoma. Med Pediatr Oncol *36*: 83-92, 2001.

28 Harrison CJ and Foroni L: Cytogenetics and molecular genetics of acute lymphoblastic leukemia. Rev Clin Exp Hematol *6*: 91-113, 2002.

29 Michalkiewicz E, Sandrini R, Figueiredo B, Miranda EC, Caran E, Oliveira-Filho AG, Marques R, Pianovski MA, Lacerda L, Cristofani LM, Jenkins J, Rodriguez-Galindo C and Ribeiro RC: Clinical and outcome characteristics of children with adrenocortical tumors: a report from the International Pediatric Adrenocortical Tumor Registry. J Clin Oncol *22*: 838-845, 2004.

Received September 25, 2008
Revised May 27, 2009
Accepted May 28, 2009