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Prognostic value of p53 expression in Wilms' tumor in children

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SUMMARY

Background: *The aim of this report was to evaluate the prognostic value and clinical correlations of p53 expression in children with Wilms' tumor.*

Material and methods: *The study comprised 61 children aged from 2 days to 13 years (median 39 months), diagnosed and treated according to SIOP and PPGGL criteria in three centers co-operating with the PPGGL. The studies were conducted on tumor tissue removed during surgery, fixed in formalin and embedded in paraffin blocks. Then 4-micron sections were evaluated by immunohistochemistry, using the peroxidase method to determine the expression of p53 in Wilms' tumor cells by means of primary monoclonal antibody NCL-p53 from Novocastra.*

Results: *The percentage of immunopositive cells in particular fragments of the tumor ranged from 0% to 70% (mean 20.4%, median 16.0%). The mean and median values enabled the children to be divided into two groups: Group A, where the percentage of cells staining with anti-p53 antibody was >20% (23 cases), and Group B, where this percentage did not exceed 20%. The expression of p53 was then evaluated in various stages of advancement and various histological types, depending on the course of the disease. In Group A, tumors at higher stages of advancement stages were more frequent ($p<0.05$), and showed a higher degree of malignancy ($p<0.06$; EFS=56.53%). In Group B, lower stages of advancement were more frequent ($p<0.05$), the degree of malignancy was lower, and the EFS was 81.58%. A discrimination test, however, showed that the determination of p53 expression in Wilms' tumor cells has moderate sensitivity (58.825%), positive prediction (43.47%), and relatively high specificity (70.45%) and negative prediction (81.57%), which means that low indexes of p53 expression have higher prognostic value.*

Conclusions: *The index of p53 expression is not an independent prognostic factor in Wilms' tumor in children, but this determination may be helpful in identifying high-risk and low-risk patients.*

BACKGROUND

Mutations or deletion of suppressor gene p53 are the most common genetic abnormalities occurring during cancerogenesis in the majority of neoplasms in humans. Gene p53, localized on the short arm of chromosome 17 (17p13), encodes nucleic 53-kDa phosphoprotein, which affects many functions in the cell, such as the induction of many genes, the regulation of the cellular cycle, and apoptosis control [1,2]. Mutated protein p53 binds to other proteins, and the heterodimer thus created enhances

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neoplasmatic transformation in various cells. In addition, abnormal protein created as a result of the mutation of one of the p53 alleles can bind to normal protein produced by the unchanged allele, inactivating it. Mutated forms of p53 can also act as oncogenes, especially since their half-life is much longer than that of other proteins [3,4].

Genome defects in the 17p13 area in neoplasms in children have been described in lymphoblastic leukemia, soft tissue sarcomas (rhabdomyosarcoma), osteosarcoma, and Wilms' tumor, but their role in Wilms' tumor has not been fully explained [5,6]. Bardessy et al. [5] found mutations of p53 in 8 cases among the 140 Wilms' tumors they studied (mainly in anaplastic tumors). However, Waber et al. [7] found no mutations in a group of 44 Wilms' tumors. Similar results were obtained by Kusafuka [6] in a group of 13 cases of Wilms' tumors. According to Velasco et al. [8], the rare occurrence of p53 mutations in Wilms' tumors shows that they are much more complex than mutations occurring in the hot places commonly seen in neoplasms in adults. Other studies, however, have shown that the lack of p53 mutation, as well as over-expression of p53, are connected with the anaplastic structure of the tumor and frequent treatment failures [9–11].

The goal of our research was to evaluate the occurrence of p53 over-expression in Wilms' tumor in children treated according to the recommendations of the PPGGL (Polish Pediatric Group for the Treatment of Solid Tumors) and the SIOP (International Society of Pediatric Oncology), and to evaluate the prognostic value of this over-expression.

MATERIAL AND METHODS

The study involved 61 children (29 girls and 32 boys), from 2 days to 13 years of age (median 39 months), treated for unilateral Wilms' tumor at three centers:

- the Surgery and Pediatric Oncology Clinic in the Institute of Pediatrics at the Medical University of Lodz (1985–1996);
- the Clinic of Pediatric Surgery in the Polish-American Institute of Pediatrics, Collegium Medicum, Jagiellonian University (Cracow);
- the Clinic of Pediatric Hematology and Oncology, Institute of Pediatrics, Medical University of Poznań (1993–1996)

Table 1. Study group characteristics (n = 61).

		Number of cases
Sex		
	female	29
	male	32
Age		
	0-6 months	6
	7-24 months	4
	> 24 months	51
Stage of advancement		
	I	26
	II N-	13
	II N+ &III	18
	IV	3
Histological type (degree of malignancy)		
	low	9
	intermediate	38
	high	11
	necrosis	3
Local recurrences, metastases		17
Deaths		10
Follow up		14–139 months (median 42 months)

All the children included in the study were diagnosed and treated according to the criteria recommended by the SIOP (International Society of Pediatric Oncology) and adopted by the Committee for Wilms' Tumor in the Polish Pediatric Group for the Treatment of Solid Tumors (PPGGL). The research group is characterized in Table 1.

The tissue samples for examination were taken from kidney tumors removed directly after diagnosis from nine children aged below 6 months, and from kidney tumors removed after initial chemotherapy from 52 children aged over 6 months.

The study was conducted on tumor tissue removed during surgery and fixed in 10% buffered formalin. The expression of p53 protein in Wilms' tumor cells was evaluated in 4-micron sections on adhesive plates, by the avidin-biotin-peroxidase method, with monoclonal antibody NCL-p53 DO7 from Novocastra. High temperature was used to reveal the antibody against protein p53, i.e. heating for 5 min in a microwave oven, a process which was repeated three times. The number of cells staining with anti-p53 antibody was calculated by ocular micrometer (type 10/20, PZO Warszawa, Poland). In all cases the percentage of cells showing p53 expression among all cells was calculated. At least

Table 2. p53: the percentage of immunopositive cells in particular components of Wilms' tumor.

p53 expression	Epithelium %	Blastema %	Stroma %
Minimum	0.000	0.000	0.000
Maximum	70.000	68.000	35.000
Mean	14.656	8.656	0.565
Standard deviation	16.620	14.690	4.445
Median	11.000	0.000	0.000

1000 cells were evaluated in each section, using a magnification of 400x [12,13].

Statistical analysis

The results we obtained were analyzed statistically using the chi-squared test, in order to compare the stage of advancement, histological type and frequency of recurrences, metastases, and deaths in Groups A and B and discrimination analysis, in order to determine if the expression of p53 could be an independent prognostic factor.

RESULTS

A positive immunoreaction was seen in 61 of studied Wilms' tumors. The percentage of cells staining with anti-p53 antibody in all three components of the tumor ranged from 0% to 70% (mean 20.3%, median 16%). The expression of p53 was highest in the epithelial elements of Wilms' tumors (70%) and in the blastema (68%), the lowest in the stroma (35%) (Table 2).

According to pathologists' recommendations [12, 13], the mean values enabled the subject children to be divided into two groups: Group A, where the percentage of cells staining with anti-p53 antibody was >20% (23 cases = 37.7%), and Group B, where this percentage did not exceed 20% (38 cases = 62.3%). In both groups, we studied the dependence between the percentage of cells staining with anti-p53 antibody on the one hand, and the stage of advancement, histological type of the tumor, and frequency of treatment failure on the other.

Expression of p53 and stage of advancement

The percentage of cells with p53 expression in particular stage of advancement is presented in Table 3. In Group A (n = 23), six children (26%) were in CS I, six (26%) in CS IIN-; ten (43.3%) in CS IIN+&III, and one case in CS IV. In Group B (n = 38) 20 children were in CS I (52.6%), 7 in CS IIN- (18.4%), 8 in CS IIN+&III (43.4%), and 3 in CS IV. Statistical analysis revealed more frequent p53 expression >20% in the tumors removed from patients in CS II N+&III in group A than in group B, and more frequent p53 expression < 20% in tumors removed from patients in CS I in Group B than in Group A, at a significance level of $p < 0.05$ (Table 3).

Table 3. Expression p53: the percentage of immunopositive cells in particular stages of advancement of Wilms' tumour.

Stage of clinical advancement		Total	
		> 20%	< 20%
I	n = 26	26%	52.6%
IIN-	n = 13	26%	18.4%
IIN+&III	n= 18	43.5%	21%
IV	n = 4	1*	3*

Table 4. Expression of p53: the percentage of immunopositive cells in particular histological types of Wilms' tumour.

Histological type/ degree of malignancy		Total	
		> 20%	< 20%
Low	n = 9	4.3%	21%
Intermediate	n = 38	60%	63%
High	n = 11	30%	10%
necrosis	n = 3	1*	2*

Expression of p53 and the histological tumor type

The percentage of cells with p53 expression in particular histological types of Wilms' tumor is presented in Table 4. In Group A (n = 23), a low degree of malignancy ('favorable outcome' according to the older histological classification scheme) was diagnosed by the pathologist in one case (4.3%); an intermediate degree of malignancy ('standard outcome' in the former histological classification) in 14 cases (60%); a high degree of malignancy ('poor outcome' in the former histological classification) in 7 cases (30%), and necrosis, which made classification difficult, in one case. In Group B (n = 38) a low degree of malignancy was diagnosed in 8 cases (21%), an intermediate degree of malignancy in 24 cases (63%), a high degree of malignancy in 4 cases (10%), and necrosis making classification difficult in 2 cases. Although a low degree of malignancy was diagnosed in Group A several times more rarely, and a high degree of malignancy three times more frequently than in Group B, the chi-squared test did not reveal the difference to be statistically significant (p=0.06).

Expression of p53 and frequency of recurrences, metastases and deaths

Among the 38 children in whom the percentage of cells staining with anti-p53 antibody was less than 20%, failure of treatment was seen in 7 cases (18.42%). Among 7 children with treatment failure, 1 died (a 5-year-old girl with focal anaplasia in Stage II, 35 months after nephrectomy), 3 children are still under treatment for recurrence, and 3 children are in second remission. However, among the 23 children in whom the percentage of cells staining with anti-p53 antibody among tumor cells was greater than 20%, treatment failure was seen in 10 children (43.47%), of whom 9 died due to disease progression, and 1 is still under treatment for a pulmonary recurrence. The results of this study show that treatment failure is significantly more frequent in tumors where the percentage of cells staining with anti-p53 antibody is 20% or more, at the level of $p<0.05$ (Table 3).

In order to evaluate the clinical value of testing to determine p53 expression, a discrimination test was done. Our analysis showed:

- indirect **sensitivity,** i.e. the probable percentage of truly positive over-expression of p53 (>20%) in the group of children with treatment failure **(58.82);**

- relatively high **specificity,** i.e. the probable percentage of truly low expression of p53 (<20%) in the group of children with incident-free survival **(70.45);**

- **prediction** (+), i.e. the probable percentage of treatment failure in children in whom tumor cells show over-expression of p53 >20% **(43.47);**

- high **prediction** (-), i.e. the probable percentage of incident-free survival in children in whom tumor cells showed a low percentage of cells staining with p53 antibody (<20%) **(81.57).**

The statistical analysis we conducted demonstrates that the determination of p53 over-expression is a useful prognostic test.

DISCUSSION

Wilms' tumor develops in the kidneys from small primary persistent focuses of blastema, the so-called nephrogenic rests, which undergo neoplasmatic transformation due to the inactivation of various genes [14]. Cytogenetic and molecular studies conducted in numerous American and European centers have shown that susceptibility to Wilms' tumor is a complex phenomenon, involving more than one genetic locus and interactions between genetic and epigenetic factors. Although the Wilms' tumor suppressor gene (WT_1) mapped in chromosome 11p13 is the best-known anti-oncogene associated with Wilms' tumor, p53 mutations have occasionally been implicated in the pathogenesis of Wilms' tumor [7,8,11,15].

This study is based on the immunohistochemical detection of p53 protein, which generally can be assumed to indicate a genetic alteration in the p53 tumor suppressor gene. Nevertheless, the results of immunohistochemical studies for the presence of p53 protein should be interpreted very carefully.

The proper interpretation of p53 protein immunopositivity still remains an unresolved question, even though most researchers now believe that the presence of staining in the majority of cells is associated with mutation and influences the clinical outcome. The cut-off for what constitutes the 'majority', hence positive staining, nonetheless remains unclear. The value accepted in various studies has ranged widely, from >10% to >75% of the tumor cells. In our work the value of the mean was set at 20%, due to the fact that pathologists suggest using mean and median values of the results obtained in order to assess the prognostic value of the proteins involved in the cellular cycle [12,13].

The studies we conducted revealed that positive immunoreaction is most frequent in epithelial cells and in blastema, and much less frequent in the stroma. This observation is consistent with that of Cheah et al. [9], who also noted positive reactions more frequently in blastemal cells. This observation confirms the common belief that blastema-type tumors have a worse prognosis.

We observed that a higher stage of advancement correlates with an increasing percentage of tumors in which cells staining with anti-p53 antibody constitute 20% or more, and with a decrease in the number of tumors in which the percentage of cells staining with anti-p53 antibody is <20%. Statistical analysis showed that an increased stage of advancement of Wilms' tumor significantly increases the number of tumors with 20% or more of cells staining with anti-p53 antibody. No significant correlation was seen between the stage of advancement and the intensity of immunopositive p53 reaction

in any of the 3 components of the tumor. The dependence between the stage of advancement and percentage of cells staining with anti-p53 antibody seen in our study seems to result from the increased aggressiveness of neoplastic cells in the higher stages of advancement, due to the loss of p53 function and the increase in replication potential [1]. We did not compare our results with those obtained by other researchers, as there are no reports on this topic in the literature.

As for rhe expression of p53 and the histological type of tumor: in the majority of tumors with a low (88.50%) or intermediate degree of malignancy (63.16%) (formerly described as favorable and standard), the percentage of cells staining with anti-p53 antibody was <20% in all three components of the tumor. A low percentage of cells staining with anti-p53 antibody was also seen in 1/3 of the tumors with a high grade of malignancy (formerly 'unfavorable'). These differences were not seen in particular elements of tumors. A high percentage of cells staining with anti-p53 antibody (>20%) was seen in all 4 tumors with diffuse anaplasia, in 2 out of 5 with focal anaplasia, and in 1 out of 2 tumors with sarcomatic stroma. These observations are consistent with those of other authors, who have noted over-expression of p53 mainly in anaplastic tumors [5,6,9,10]. The high percentage of cells staining with anti-p53 antibody (>20%) in high malignancy tumors (mainly those with diffuse anaplasia) is probably connected with the appearance of cells which have lost the p53 function and become aneuploid with various chromosomal transformations. It has been suggested that a high level of aneuploidia is responsible for resistance to chemotherapy and poor prognosis [14–16]. It is thought that diffuse anaplasia – diffuse dissemination of anaplastic cells – is connected with a poor prognosis, and focal anaplasia in Wilms' tumors without extra renal diffusion (stage I) with good prognosis [16–18]. Although it has been noted that an increase in the malignancy of Wilms' tumor is connected with an increase in the percentage of immunopositive cells, this observation has not been confirmed statistically. It may be dependent on the number of cases in the research groups. It has been suggested that the amount of p53 expression by Wilms' tumors may be linked to tumor aggressiveness associated with anaplasia [5,10,16]. A speculative explanation may be that these more sinister tumors with unfavorable histology show more rapid and active production or slower elimination of mutant protein. This may in turn result in increased inhibition of oncogenic suppression or heightened oncogenic activity, as suggested by increased aggressiveness.

The analysis of the disease course in children with Wilms' tumor shows significantly more frequent occurrence ($p<0.005$) of relapses, metastases, and death in children in whom the tumors cells show an over-expression of p53>20. Treatment failures mainly involved children with Wilms' tumors the in high and intermediate degree of malignancy at the stage of advancement CS IIN+&III. Our observations are consistent with those of other authors [5,6,9–11], who have observed over-expression of p53 in anaplastic Wilms' tumors. The presence of p53 over-expression also in anaplastic tumors removed after initial chemotherapy suggests that these tumors are resistant to currently used treatment methods. It should be noted that there were no failures of treatment in children with Wilms' tumor in the low or intermediate degree of malignancy and in Stage I of advancement, where the expression of p53 was <20%. This may suggest a favorable prognosis for this group.

The discrimination analysis revealed that although over-expression of p53 is connected with a higher stage of advancement and significantly more frequent treatment failures, it is not an independent prognostic factor. This is demonstrated by the low sensitivity (58.82%) and low positive prediction (43.47%) of this determination. However, the relatively high specificity (70.45%) and negative prediction (81.57%) may suggest that a low percentage of immunopositive cells may help to classify a child in the low risk group.

CONCLUSIONS

The index of p53 expression is not an independent prognostic factor in Wilms' tumor in children, but it may be helpful in the identification of high risk and low risk patients.

REFERENCES:

1. *Carson DA: Cancer progression and p53. Lancet, 1995; 346: 1009-1011*
2. *Hall PA, Lane DP: p53 in Tumor Pathology: Can we trust immunohistochemistry? Revisited J Pathol, 1994; 172: 1-4*
3. *Hollestein M, Sidransky D, Vogelstein B, Harris CC: p53 Mutations inHuman Cancers. Science, 1991; 253: 49-53*
4. *Soussi T: Le gene suppresseur de tumeur p53. Bull Cancer, 1993; 80: 96-101*
5. *Bardessy N, Falkoff D, Petruzzi MJ et al: Anaplastic Wilms' Tumor: A subtype displaying poor prognosis harbours p53 gene mutations. Nature Genet, 1994; 7: 91-97*

5. *Kusafuka T, Fukuzawa M, Oue T et al: Mutation analysis of p53 gene in childhood malignant solid tumors. J Pediatr Surg, 1997; 32(8): 1175-1180*
6. *Waber PG, Chen J, Nisen PD: Infrequency of Ras, p53, WT1, or Rb gene alterations in Wilms' tumors. Cancer Res, 1993; 72(12): 3732-3738*
7. *Velasco S, D'Amico D, Schneider NR et al: Molecular and cellular heterogeneity of Wilms' tumor. Int J Cancer, 1993; 53: 672-679*
8. *Cheah PL, Looi LM, Chan LL: Immunohistochemical expression of p53 protein in Wilms' tumor: a possible association with the histological prognostic parameter of anaplasia. Histopathology, 1996; 28: 49–54*
9. *Lahoti C, Malkin D, Yeger H: Immunohistochemical detection of p53 in Wilms' tumors correlates with recurrence and metastasis. Med Pediatr Oncol, 1996; 27: 503*
10. *Lemoine NR, Hughes CM, Cowell JK: Aberrant expression of the tumor suppressor gene p53 is very frequent in Wilms' tumors. J Pathol, 1992; 168: 237-242*
11. *Linden MD, Torres FX, Kobus J, Zarbo RJ: Clinical application of morphologic and immunocytochemical assessment of cell proliferation. Am J Clin Pathol, 1992; 97(5 suppl. 1): 4-13*
12. *Taylor CR, Shi S-R, Chaiwun B et al: Strategies for improving the immunohistochemical staining of various intranuclear prognostic markers in formalin paraffin-sections; androgen receptor; estrogen receptor; progesteron receptor; p53 protein; proliferating cell nuclear antigen and Ki--67 antigen revealed techniques. Hum Pathol, 1994; 25: 263-270*
13. *Schmidt D, Beckwith JB: Histopathology of childhood renal tumors. Hematology/ Oncology Clinics of North America, 1995; 9(6): 1179-1200*
14. *Hastie N: The genetics of wilms' tumor: a case of disrupted development. Annu Rev Genet, 1994; 28: 523-558*
15. *Beckwith JB, Zuppan CE, Browning NG et al: Histological analysis of aggressiveness and responsiveness in Wilms' tumor. Med Pediatr Oncol, 1996; 27: 422-428*
16. *Vujanic GM, Weirich A, Sandstedt B et al: Anaplastic Wilms' Tumors – The SIOP 9 trial and study experience with the new definitions. Med Pediatr Oncol, 1997; 29: 346-347*
17. *Vujanic GM, Harms D, Sandstedt B et al: New definitions of focal and diffuse anaplasia in Wilms' tumor: the International Society of Pediatric Oncology (SIOP) experience. Med Pediatr Oncol, 1999; 32: 317-323`*