

# Evaluation of TP53 Gene Expression in Patients with Childhood Cancer in Northeast Santa Catarina, Brazil

## *Avaliação da expressão do gene TP53 em pacientes com câncer infanto-juvenil no nordeste de Santa Catarina, Brasil*

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### ABSTRACT

**Introduction:** In Brazil, 8,000 new cases of childhood cancer are estimated each year, whose causes are still little known, although some have genetically determined factors. Approximately 70% of human cancers have alterations in the TP53 gene, which encodes the protein responsible for inhibiting the disordered growth of cells exposed to injuries. However, the frequency of alterations in the expression of TP53 in childhood cancers in Brazil remains poorly known.

**Objective:** To evaluate the expression of TP53 gene in patients with childhood cancer in northeastern of Santa Catarina, Brazil. **Materials and Methods:** Retrospectively, 282 patients diagnosed with cancer between 2005 and 2015 in Joinville were included. TP53 expression was evaluated by immunohistochemistry using a score based on the intensity and percentage of stained cells.

**Results:** The p53 protein was positive in 25.2% of cases, with no difference between sexes. Considering the five main groups of tumors in the sample, the expression was positive in 31.8%, 27.3%, 20%, 17.2% and 5.9% of lymphomas, neuroblastomas, neuroblastomas, tumors of the Central Nervous System and leukemias, respectively.

**Conclusion:** The prevalence of TP53 expression was evaluated in different childhood cancers in the northeastern of Santa Catarina. Positivity was higher among lymphomas and lower in leukemias, but with no significant difference among the five most frequent tumors. Further studies that allow correlation with aggressiveness and disease evolution are required.

**Key words:** childhood cancer; gene TP53; immunohistochemistry.

### INTRODUCTION

Cancer is a term that encompasses more than 100 different types of malignant diseases that have in common the disordered growth of cells, which can invade adjacent tissues or distant organs<sup>(1)</sup>. In the Brazilian territory, an approximate number of one million cases are observed annually, considering the adult and children's public<sup>(2)</sup>.

The main types of childhood cancer are leukemias, in first place in incidence, followed by central nervous system (CNS) tumors, lymphomas, neuroblastomas, neuroblastomas and bone

and muscle tumors<sup>(3)</sup>. While adult tumors are, in general, related to exposure to several defined risk factors, the causes of pediatric tumors are still poorly understood, although in some specific types there is already scientific basis that they are genetically determined<sup>(4,5)</sup>.

Progress in childhood cancer treatment has grown significantly in the last four decades, as in the mid-1970s, the 5-year survival rate was only 58%<sup>(1)</sup>. Currently, it is estimated that around 70% of children with cancer can be cured and more than 84% of children with cancer survive 5 years or more, adding to a better quality of life during the entire period of treatment<sup>(6-8)</sup>.

The treatment of childhood cancer is based on a complex rationale, consisting of chemotherapy, radiotherapy and a surgical approach, in addition to the assistance of a specialized multidisciplinary team in a tertiary hospital, with a significant economic impact, considering the hospital costs with providers of direct and indirect services<sup>(6-10)</sup>.

Even with the progressive improvement in the cure rate, mortality still stands out as significant in the country, corresponding to the eighth position among the causes of death in children aged 0 to 4 years, as well as being the main cause of death in the age group of 5 to 19 years in 2018<sup>(11)</sup>. With the intention of further improving cure rates, different studies have focused on one of the primordial genes identified in association with cancer development - TP53, which plays a role in tumor suppression. Nucleotide alterations increase the propensity to generate cancer cells in relation to the general population<sup>(12)</sup>, noting that approximately 70% of human cancers have some alteration in the function of TP53<sup>(12,13)</sup>. Therefore, a still current challenge for pediatric oncology is to know the molecular biology of the tumors in this population, as well as to assess the significance of the expression of the TP53 mutation.

The present study aimed to investigate the local child-juvenile population regarding the prevalence of TP53 expression via immunohistochemistry, evaluating its distribution according to demographic characteristics and histological type.

## MATERIALS AND METHODS

This was a cross-sectional epidemiological study, with laboratory analysis of retrospectively selected cases, aiming to investigate the prevalence of TP53 gene expression in underage patients in Joinville, one of the main children and youth cancer treatment centers in the state of Santa Catarina, Brazil.

The study was carried out using the database and biological materials stored at the *Centro de Diagnósticos Anátomo-Patológicas* (CEDAP), a pathological anatomy laboratory located in the same city, accredited by the American College of Pathologists and the National Accreditation Organization. All patients under 18 years of age diagnosed with cancer in the period between 2005 and 2015 were included in the study, and the assessment of TP53 expression was correlated with the demographic characteristics of sex and age and the histological type of the tumor.

The study was approved by the Research Ethics Committee of the University of the Region of Joinville (UNIVILLE) (Opinion No. 3,619,240).

**Immunohistochemical Analysis:** From the identification of infant and juvenile cases diagnosed with cancer, new sections with a thickness of 3 µm for the preparation of slides were made in the samples stored in paraffin blocks. Antigen retrieval was performed using the PT-Link equipment (Dako, Glostrup, Denmark), at high pH, for 20 minutes at 95°C. The immunohistochemistry technique was performed automatically with the “Autostainer Link 48” equipment (Dako), using the ready-to-use mouse monoclonal antibody anti-p53 clone DO-7 (Dako). To block the peroxidase, a 3% hydrogen peroxide solution (Dako) was used. Then, the slides were incubated in the solution “EnVision FLEX/HRP Polymer” (Dako) for 20 minutes, while “EnVision FLEX DAB Chromogen” (Dako) was used for 5 minutes for development. Slides were counterstained with Gill’s hematoxylin.

Colon carcinoma was used as a positive control for p53 protein, while the negative control was performed without the use of primary antibody, according to the usual immunohistochemical procedure of the aforementioned laboratory. The method of reading and interpreting the immunohistochemistry results was based on Queiroz (2006)<sup>(14)</sup> and Rocha et al. (2004)<sup>(15)</sup>. Slides were examined blinded to patient demographics and clinical data and independently by two CEDAP pathologists experienced in analyzing p53 technique results. The percentage of positive cells and the intensity of staining in each sample were considered. For the stratification of the percentage of positive cells, a scale from 0 to 3 was established, with 0 to 10% of stained cells classified as “0”, >10 to 25% as “1”, >25 to 50% as “2” and >50% as “3”. In turn, the staining intensity was graded on another scale from 0 to 3: negative result (absence) was classified as “0”, weak as “1”, moderate as “2” and strong as “3”. The final score for each case was obtained by multiplying the classification result derived from the percentage of stained cells by the classification result resulting from the analysis of staining intensity<sup>(16)</sup>. In the final analysis, a score less than 3 was considered negative, while scores equal to or greater than 3 were considered positive.

**Statistical Analysis:** Continuous variables were presented as mean and standard deviation, while categorical variables were presented as number of cases and percentage. The interobserver variability, both for the staining intensity index and for the percentage amount of stained cells, was evaluated by Cohen’s kappa test. The chi-square test of independence was applied to evaluate the samples regarding the variables intensity, quantity (percentage) and final immunohistochemical score in relation to the distributions between tumor types, sexes and age groups (below 2 years = infants, 3 to 5 years old = preschoolers, 6 to 11 years old = school children and over 11 years old = teenagers). The decision significance level was 0.05 (5%) and the statistical

analysis was supported by the Statistical Package of Social Science (SPSS) version 26.0.

## RESULTS

A total of 282 patients were included in this study. Of the total regarding sex, 148 (52.5%) were men. As for the age of the patients, these were distributed in 45 cases (16.0%) of infants, 45 (16.0%) of preschool children, 67 (23.7%) of schoolchildren and 125 of adolescents, presenting in mean  $9.5 \pm 5.3$  years (Table 1).

TABLE 1- Results of immunohistochemical expression of the TP53 gene in tumors of children and adolescents

Demographic characteristics and oncological diagnosis	Sample studied, N (%)	Immunohistochemical expression		P
		Positive, n (%)	Negative, n (%)	
Total	282	71 (25.2)	211 (74.8)	
Age, years (mean $\pm$ SD)	$9.5 \pm 5.3$	$9.5 \pm 5.3$	$9.4 \pm 5.3$	0.04
Infants	45 (16.0)	16 (35.6)	29 (64.4)	
Preschoolers	45 (16.0)	5 (11.1)	40 (88.9)	
Schoolchildren	67 (23.7)	15 (22.4)	52 (77.6)	
Adolescentes	125 (44.3)	35 (28.0)	90 (72.0)	
Gender				0.37
Female	134 (47.5)	37 (27.6)	97 (72.4)	
Male	148 (52.5)	34 (23.0)	114 (77.0)	
Major childhood tumors in the series	160 (56.7)	35 (21.9)	125 (78.1)	0.056
Lymphomas	66 (23.4)	21 (31.8)	45 (68.2)	
Leukemias	34 (12.1)	2 (5.9)	32 (94.1)	
CNS tumors	29 (10.3)	5 (17.2)	24 (82.8)	
Neuroblastomas	20 (7.1)	4 (20.0)	16 (80.0)	
Nephroblastomas	11 (3.9)	3 (27.3)	8 (72.7)	
Others	122 (43.3)	36 (29.5)	86 (70.5)	

CNS: Central Nervous System

Most patients ( $n = 160$ ; 56.7%) corresponded to one of the five main types of childhood and juvenile tumors, while 122 patients (43.3%) had other histological specifications. Thus, in the series detailed in the study, the five main tumors in terms of frequency were lymphomas ( $n = 66$ ; 21 being Hodgkin, 42 Non-Hodgkin and 3 unclassified), leukemia ( $n = 34$ ; 16 being acute lymphoblastic leukemia, 14 acute myeloblastic leukemias and 4 unclassified), CNS tumors ( $n = 29$ ; 21 astrocytomas, 3 medulloblastomas, 3 ependymomas and 2 retinoblastomas), neuroblastomas ( $n = 20$ ) and nephroblastomas ( $n = 11$ ) (Table 1).

For each case, the immunohistochemical evaluations were carried out independently by two pathologists regarding

the percentage of positive cells and staining intensity, being concordant: the interobserver variability was minimal, as the kappa test showed an almost perfect agreement between pathologists for both variables ( $k=0.873$ ; 95% CI 0.81-0.94;  $p<0.001$  and  $k=0.877$  95% CI 0.817-0.937;  $p<0.001$ , respectively). Of the 282 cases evaluated, 71 individuals (25.2%) had a positive expression for p53, while the other 211 had a negative test. The distribution of positive expression of p53 protein in terms of sex showed 52.1% ( $n = 37$ ) of female cases and 47.9% ( $n = 34$ ) of male cases, with no significant difference.

Considering the five main tumors in terms of frequency in the series, 2 (5.9%) of the 34 patients diagnosed with leukemia had a positive p53 result. As for CNS tumors ( $n = 29$ ), 5 (26.9%) were positive for p53, regardless of the histological subgroup. In turn, among the 66 cases of lymphoma in the series, the positivity of the expression of the TP53 gene was 31.8% ( $n = 21$ ). Among neuroblastomas ( $n = 20$ ) and nephroblastomas ( $n = 11$ ), the expression was positive in 4 (20%) and 3 (27.3%) cases, respectively. There was no significant difference in the distribution of positivity for the p53 marker among the five groups of tumors considered (Table 1).

The chi-square test of independence was performed to verify the association between the age groups in the studied sample with the expression of p53, identifying a possible significant difference between the groups ( $p = 0.04$ ). Additionally, there was no difference in expression between the sexes (Table 1).

## DISCUSSION

The natural history of cancer indicates that the clinical course of the disease and survival vary from patient to patient. This variation is determined by several factors that are not yet fully understood in most tumors, such as childhood cancers<sup>(1,7,17-19)</sup>.

One of the ways to advance in the scenario of pediatric oncology practice is the elucidation of the carcinogenesis process and the identification of biomarkers for drug development, early diagnosis, determination of prognosis and prediction of response to treatment<sup>(1,7-10)</sup>. Despite advances in the knowledge of genetic mechanisms, the role of the TP53 gene as a participating factor in the genesis of cancer, as well as its clinical relevance and importance in prognosis, remains controversial. Some authors credit changes in TP53 with a worse prognosis<sup>(10, 20,21)</sup>.

In this work, focusing on evaluating the immunohistochemical expression of the TP53 gene in patients with childhood cancer in the northeast of Santa Catarina, southern Brazil, three indices were performed - staining intensity, percentage of stained cells

and score (product) final - based on archived samples since 2005, which were reprocessed for study purposes. Thus, we sought to analyze the prevalence of TP53 expression, as described by similar previous studies carried out in other populations<sup>(15,16,22-24)</sup>.

The analysis of immunohistochemical expression based on a score derived from the intensity and percentage of labeled cells is a valid method used by several authors, with variations in relation to the percentage of stained cells to consider TP53 mutated or not, ranging from just one cell up to 25% of the cells in the analyzed field. In turn, the assessment of intensity varied between descriptors indicated as “very weak or not very intense” to “very strong or very intense”. However, most studies use a cut of 10% of stained cells as a minimum to show positivity of p53 expression, while the intensity classification varies from “absent” to “strong or intense”, which were the limits adopted by the present study<sup>(15,16,23,25-27)</sup>.

In our study, the positivity of TP53 expression was only 25.2%. The result obtained was below the corresponding values found in the literature, which indicate values between 50% and 70% derived from neoplasms and different techniques for evaluating expression<sup>(6,28-31)</sup>. The discrepant finding may be related to the increasing technological sophistication of diagnostic methods with greater sensitivity, such as those based on the Polymerase Chain Reaction (PCR)<sup>(20,21)</sup>. For example, Marques (2009)<sup>(10)</sup>, in his study of 41 adult patients with esophageal cancer, identified 69.3% of TP53 gene expression when evaluating it via PCR.

The positivity of immunohistochemistry in the evaluation of p53, in the absence of detectable mutation of the coding gene, can be explained in several ways. The wild-type p53 protein can accumulate at levels sufficient to be detected by immunohistochemistry in both normal and neoplastic cells, because its accumulation more broadly reflects the cellular environment than simply the intrinsic structure of the protein. On the other hand, immunohistochemistry negativity in tumors containing a mutation may reflect the lack of sensitivity or the lack of accumulation of the p53 protein itself. The latter can occur when protein production is blocked or truncated as a result of a mutation, particularly as a result of deletions, splicing mutations and nonsense mutations<sup>(32,33)</sup>.

The difficulties in associating the status of the TP53 gene with the biological properties of cancer cells and the sensitivity of these cells to different drugs could be explained, in part, by the expression of different isoforms of the gene. TP53 contains an alternative internal promoter in intron 4 and can transcribe 9 isoforms that have different subcellular locations. Thus, the use of a panel of anti-p53 antibodies in immunohistochemistry may result in different patterns in the same tumor sample<sup>(33)</sup>.

Monoclonal antibodies DO-1 and DO-7 recognize p53, p53 $\beta$  and p53 $\gamma$ , but do not recognize the other isoforms of the p53 protein. In order to obtain a better understanding of p53 immunodetection, a PCR-associated reverse transcription could be used, aiming at the detection of different p53 isoforms. However, such assays require high quality RNA, which makes the process more difficult<sup>(21,32-34)</sup>.

Alternatively, the presence of a TP53 mutation may not be detected by molecular analysis. Highly conserved regions of the gene (exons 5-8) are generally chosen for analysis, as they contain the vast majority of known mutations. Mutations that occur outside these exons may not be detected, but are less frequent<sup>(35)</sup>. More recently, molecular studies have been extended to exons 4, 9 and 10 and it has been observed that these contain a considerable number of mutations (about 15%). The direct sequencing of the TP53 gene after PCR amplification remains the “gold standard” of molecular analysis, but not very accessible to most pathology laboratories<sup>(33,34)</sup>.

Additional complications in evaluating p53 protein immunodetection include variations in techniques and patterns of positivity interpretation. In any case, it is likely that different patterns of TP53 expression have a different biological basis and that these differences may have clinical relevance. Its detection is normally located in the nucleus of malignant cells, having no detectable expression in normal cells. The mutation is normally accompanied by positivity in a large proportion of the malignant cell population, but the immunodetection patterns are rarely homogeneous. Thus, the standardization of both laboratory methodology and reading interpretation are essential for the routine use of immunohistochemistry<sup>(33,34)</sup>.

False negatives can occur and are usually due to the use of only one antibody and/or improper use of the material, either formalin-fixed or material frozen for a long time. There are a number of non-controllable variables in tissue processing that could explain the different immunodetection patterns between individual blocks, such as, for example, the time between tissue removal and fixation, the size of the sample to be fixated, and the time elapsed since tissue fixation and temperature of the state of submersion in paraffin. All these factors have a direct impact on the quality of antigen preservation<sup>(35)</sup>.

In our study, the distribution of positive expression of p53 protein in terms of sex showed 52.1% (n = 37) of female cases and 47.9% (n = 34) of male cases, which is similar to that described in the literature, which points to a small, non-significant global prevalence of males<sup>(35-41)</sup>.

Leukemias are the most common type of cancer in children

and adolescents<sup>(7)</sup>; however, in our study, we evaluated the expression of p53 in bone marrow biopsies, which is not the most suitable method for such diagnosis, with immunophenotyping by flow cytometry being the gold standard method and conventionally used<sup>(35,36)</sup>. In our series regarding this type of cancer (n = 34), the positive expression occurred in only 2 cases (5.9%). Meanwhile, Bainer et al. (2017)<sup>(42)</sup>, using immunohistochemistry with an anti-TP53 antibody identical to that of the present study, demonstrated a positivity of 23% among individuals aged 0 to 18 years in a total of 465 cases at St. Jude Children's Research Hospital, in the USA. In turn, Demir et al. (2020)<sup>(27)</sup>, using the same technique in 62 pediatric patients at Aprea Therapeutics, in Sweden, found 6.5% of positive cases. On the other hand, using the PCR technique, Ding et al. (2017)<sup>(43)</sup> found 11.6% positivity in 154 patients (0 to 18 years) at the Cancer Science Institute in Singapore.

As for CNS tumors, different authors indicate that TP53 mutations are usually present in 20 to 75% of cases, using immunohistochemistry and anti-TP53 monoclonal antibody as a technique. Pope et al. (2007)<sup>(44)</sup>, in their series of patients up to 12 years of age with glioblastoma, in Curitiba, found 75% of positivity. Kwon et al. (2020)<sup>(45)</sup>, evaluating 89 patients between 0 and 80 years old diagnosed with glioblastoma, found 44.4% positivity in immunohistochemistry for p53 at Samsung Medical Center, South Korea. In turn, Nweke et al. (2021)<sup>(46)</sup> observed positivity corresponding to 21% of cases in 81 patients, including children and adults, with a mean age of 30.6 years, at the Tertiary Nigerian Hospital, Nigeria. Finally, Uppar et al. (2019)<sup>(47)</sup>, also using immunohistochemistry, identified 69% positivity in 29 samples of patients aged between 3 and 18 years at the National Institute of Mental Health, India. In our sample, we found 26.9% of immunopositivity in CNS tumors (n = 29), similar to the literature.

Among the lymphoma specimens in the series studied (n = 66), positive expression of TP53 was observed in 31.8% of the cases. Genet et al. (2019)<sup>(48)</sup>, using fluorescent in situ hybridization, identified positivity in 32% of 29 patients with Non-Hodgkin Lymphoma (NHL) in China. Using PCR, Ichikawa et al. (1997)<sup>(49)</sup> observed expression of the TP53 mutation in 22% of 102 samples analyzed at the Grants-in-Aid for Cancer Research, Japan, including children and adults. In turn, using immunohistochemistry with anti-TP53 monoclonal antibody, Klumb et al. (2003)<sup>(50)</sup> found 36% of positive cases in 49 patients (0 to 15 years old) with NHL, at the Department of Medical Biochemistry at the Federal University of Rio de Janeiro, Brazil. Also employing immunohistochemistry, Magluta, Klumb (2008)<sup>(51)</sup>, at the Instituto Nacional do Câncer, Rio de Janeiro, reported 30% positivity in patients diagnosed with Burkitt's Lymphoma. Our results regarding lymphomas are very

close to those observed in other populations.

For our neuroblastoma cases (n = 20), expression was positive in 4 cases (20%). Apparently, there is indeed a low correlation between mutations in TP53 and this type of cancer. Oh et al. (2019)<sup>(28)</sup>, in a review article and without delimiting the technique and age group of patients, reported 1.8% of cases of p53-positive neuroblastomas. Seidinger et al. (2015)<sup>(52)</sup>, evaluating the expression through PCR, identified 8.4% positivity among 178 patients, aged between 0 and 18 years, in a treatment center in Campinas, Brazil. The apparent high percentage of immunodetection in our study may have been influenced by the relatively small number of cases for this type of cancer in the series and, thus, inferences regarding mutations in TP53 are impaired.

Among our nephroblastoma cases (n = 11), p53 expression was positive in 3 cases, representing 27.3%. He et al. (2020)<sup>(53)</sup>, using miRNA assessment, identified 2.3% of positive cases among children under 10 years of age (n = 178) at Youjiang Medical University For Nationalities Affiliated Hospital, Baise, China. Wang et al. (2020)<sup>(54)</sup>, among 183 patients with nephroblastoma, regardless of age, reported 57% of positive cases at the Yuying Children's Hospital of Wenzhou Medical University and Guangzhou Women and Children's Medical Center, using genotyping to identify polymorphisms of interest in the expression of the TP53 gene.

For the early diagnosis of childhood cancer, in addition to recognizing its signs and symptoms, actions are needed that imply the joint action of health organizations and professional training bodies, in order to promote comprehensive care, ensuring accessibility, comprehensiveness and resolution<sup>(1,7,55-57)</sup>. Furthermore, the definition of a subgroup of predominantly immuno-positive p53 tumors could bring to light a better understanding of the tumor and, consequently, new possibilities for approaches. However, for such clinical correlations, it is necessary to incorporate diagnostic methods into daily practice that identify genetic abnormalities, in addition to conventional histopathology<sup>(55)</sup>.

Some limitations of the present study should be highlighted. Initially, the limited size of the sample, due to the fact that the study was conducted in a single center, made it impossible to correlate the results obtained with the histological sub-classification within each type of tumor, even considering the most frequent ones. That is, the results found should not be extrapolated directly to other locations and pediatric oncology or pathology services. Additionally, due to the retrospective design applied to the study, it is possible that the long storage time of the samples and consequent gradual degradation had a negative impact on a

portion of potentially false negative results.

## CONCLUSION

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The expression of the TP53 gene, investigated by immunohistochemistry, was evaluated in terms of prevalence in a population with childhood cancer at a reference diagnostic center

in northern Santa Catarina. Positivity was confirmed in 25.2% of the cases, with no difference between the sexes. Immunodetection of p53 protein was higher among lymphoma cases (31.8%) and lower among leukemia cases (5.9%). The meaning of TP53 expression in different tumors, with a view to improving the diagnosis and treatment of childhood cancers, demands studies that combine more precise analytical methods and allow a correlation with the aggressiveness and evolution of the disease.

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