RESEARCH ARTICLE

The *p16* Immunostaining Predicts the Risk of Recurrence in Prostate Cancer

Sara Lourrane Carneiro de Andrade Cavalcante[1], Paulo Goberlânio de Barros Silva[1]*, Carlos Gustavo Hirth[1], Ingrid Kellen Sousa Frederico[2], Cristiana Libardi Miranda Furtado[2], Cláudia do Ó Pessoa[2], Giulianna Aparecida Vieira Barreto[1], Lúcio Flávio Gonzaga Silva[1]

Abstract

Objective: This study aimed to investigate the influence of *p16* immunohistochemical expression on the biochemical recurrence rate of pT2-pT3 prostate cancer. **Materials and Methods:** A total of 488 pT2-pT3 stage prostate adenocarcinomas undergoing radical prostatectomy were included in this study. Following a review of Gleason classification and retrieval of sociodemographic and clinicopathological data, as well as the date of last consultation and biochemical recurrence, immunohistochemistry for *p16* was performed. Data were associated using the chi-square test, Fisher's exact test, and multinomial logistic regression model. **Results:** A total of 432(94.5%) cases showed positivity for *p16* with an average of 37.38±27.32% positive cells and a mean histoscore of 2.70±2.24. A total of 117 (18.4%) patients experienced biochemical recurrence within three years, which was directly associated with high preoperative PSA (p=0.007), positive surgical margins (p<0.001), pT3 staging (p<0.001), nodal involvement (p<0.001), Gleason score > 3+4 (p<0.001), <50% positivity for *p16* (p=0.035), and histoscore *p16* =<3 (p=0.004). In multivariate analysis, Gleason score > 3+4 (HR = 3.08 (95% CI = 1.69-5.62), positive surgical margins (HR = 2.93 (95% CI = 1.70-5.04), and histoscore *p16* =<3 (HR = 2.49 (95% CI = 1.17-5.32) were predictors of biochemical recurrence within three years. **Conclusion:** *p16* immunostaining, along with classical features such as Gleason Score and surgical margin involvement, are significant predictors of biochemical recurrence in pT2-pT3 prostate tumors.

Keywords: Human Papillomavirus- Prostate Neoplasms- Recurrence Time

Introduction

Prostate cancer is the first cancer in incidence among men and the fourth most prevalent (7.3%) [1]. In 2020, an estimated 1.4 million new cases were reported, accounting for 15.2% of all cancer types in the male population [1]. The highest incidence rates of prostate cancer are found in Northern Europe, Western Europe, the Caribbean, and Oceania [2, 3]. In Brazil, it is estimated that there will be 71,730 new cases of prostate cancer for each year of the triennium 2023, 2024, and 2025, corresponding to a conjectured risk of 67.86 new cases per 100,000 men [1].

Prostate cancer pathogenesis is related to family history, hereditary genetic factors (Lynch syndrome and mutations in BRCA1 and BRCA2) [1, 4], smoking, obesity [5, 6], and exposures to aromatic amines, arsenic, and petroleum products [1]. However, the involvement of microbiomes has been associated with various types of cancers [7].

HPV infections are directly associated with many tumors. Its pathogenesis is centered on the infection of epithelial cells, which affect skin and mucous membranes, being one of the primary etiological agents of cervical cancer, oropharyngeal cancer, anogenital tract cancers, skin cancers, and oral mucosal cancers (OPSCC) [8]. Since HPV manifestation is prevalent, primarily due to its transmission through sexual contact, organs associated with sexual activity, such as the prostate, may be affected and play a role in oncological pathogenesis [9, 8].

HPV-related tumors, such as cervical and oropharyngeal carcinomas, exhibit tumorigenesis containing HPV sequences identified by *p16* immunohistochemical expression [7]. Additionally, HPV-positive patients manifest clinical characteristics different from those negative for HPV [7, 10]. HPV-positive tumors have better differentiation and higher antigenicity than HPV-negative

*[1]Postgraduate Program in Oncology, Haroldo Juaçaba Hospital, Ceará Cancer Institute (ICC), Brazil. [2]Department of Pharmacology, Postgraduate Program in Pharmacology, Federal University of Ceará, Brazil. *For Correspondence: paulo_goberlanio@yahoo.com.br*

tumors [11].

In the pelvic region, HPV presence encompasses 85% of anal cancers and 50% of vaginal, vulvar, and penile cancers [12]. Therefore, this study aimed to characterize *p16* immunohistochemical expression in prostate tumors and evaluate its influence on biochemical recurrence rate.

Materials and Methods

Study design and ethical aspects

This is a retrospective observational cohort study conducted at the Haroldo Juaçaba Hospital/Cancer Institute of Ceará (Brazil) in collaboration with the Federal University of Ceará (UFC), including patients with pT2, pT3a, and pT3b prostate tumors who underwent radical prostatectomy for prostate cancer from January 2009 to December 2016, performed at the Haroldo Juaçaba Hospital/Cancer Institute of Ceará.

The Ethics and Research Committee of the Haroldo Juaçaba Hospital/Cancer Institute of Ceará approved the study in 2020 under protocol number 71673317000005528.

Study participants and inclusion and exclusion criteria

Paraffin-embedded blocks of prostate adenocarcinoma resections from the Livino Pinheiro Laboratory of the Haroldo Juaçaba Hospital/Cancer Institute of Ceará from January 2009 to December 2016 were included. Patients who underwent neoadjuvant therapy, patients diagnosed with another malignancy before or concurrently with prostate adenocarcinoma diagnosis, patients with metastases diagnosed before surgery through scintigraphy, computed tomography, and/or magnetic resonance imaging, patients without a minimum clinical follow-up record of two months, and patients without archived material in paraffin blocks and histological slides for review and preparation of tissue microarrays (TMA) were excluded.

Case survey and Tissue Microarray construction

Initially, anatomopathological reports and physical/electronic medical records were reviewed to screen for cases of prostate cancer within the inclusion criteria. The areas of each component were identified on the histological slide with circles using a permanent marker pen.

Subsequently, the TMAs were constructed using material embedded in paraffin, based on the markings made on the histological slides, using 2.0 mm punches directed at the areas of interest. Specifically, if a Gleason pattern was represented (3, 4, or 5), two punches were taken: one for the non-neoplastic glandular tissue and another for the neoplastic tissue. If there was a representation of two Gleason patterns (3 and/or 4 and/or 5), three punches were taken, and when there were three Gleason patterns (3, 4, and 5), four punches were taken.

Immunohistochemical processing and analysis

The TMA blocks were cut into three micrometers, with one section of each block stained with hematoxylin and eosin (HE) and the remaining sections undergoing immunohistochemistry for *p16*/INK4A (Merck®). The immunohistochemical reaction was performed using the streptavidin-biotin-peroxidase technique. Antigen retrieval was carried out with a 0.1M tris-EDTA solution pH 9.0 in a water bath at 97°C for 30 minutes. Subsequently, endogenous peroxidase blocking was done with 3% hydrogen peroxide diluted in PBS, followed by incubation for one hour with the aforementioned primary antibody *p16*/INK4A (Merck®), Clone SRP3134, 1:200). After the specified incubation time with the primary antibody, the slides were washed with PBS and then incubated with biotinylated antibody for 30 minutes [13].

After washing, the slides were incubated with avidin-biotin peroxidase conjugate or Envision® system for 30 minutes. Visualization was achieved by incubating with 3,3'-Diamino-benzidine (DAB) (Abcam®) and counterstaining with 7% Harris hematoxylin for 10 seconds. Following staining and counterstaining, the slides were rinsed in running water, dehydrated, cleared, and mounted with Entellan®. The positive control was a sample of oropharyngeal cancer known to be *p16*+. The negative control was performed by omitting the primary antibody.

The immunohistochemical reactions were blindly assessed by a pathologist with over 10 years of experience. The first phase of the analysis involved observing the HE-stained sections and confirming the presence of non-neoplastic tissue and the sampled Gleason patterns in each fragment [14].

Analyzing the studied markers, a minimum of 100 cells for each compartment was considered representative. The percentage of marked cells (0: no marked cells; 1: 1-25% of marked cells; 2: 26-50% of marked cells; 3: 51-75% of marked cells; 4: 76-100% of marked cells) and the intensity of the reaction (3: strong when easily visible at 40x magnification; 2: moderate when easily visible at 100x magnification; 1: weak when visible at 400x magnification; 0: negative when no staining was present [15] were considered. The analyses were performed using an Eclipse E200® microscope, Nikon. Subsequently, the extent and intensity of staining were multiplied to calculate the histoscore [16].

Clinical-prognostic data collection

The patient's medical records were reviewed to collect the following information: Age, preoperative PSA (categorized as ≤ 10 and >10 ng/ml [17], Margin status, Pathological staging, Lymph node involvement, Biochemical persistence, Need for adjuvant therapy, Biochemical recurrence within 3 years, Salvage therapy, and Development of metastasis.

The Gleason pattern, categorized as 3, 4, or 5, was determined from the review of histological slides of prostatectomies and TMA samples [18, 19]. The surgical Margin status, obtained from the histological slide review of prostatectomies, was categorized as either straightforward or involved, following the guidelines of the International Society of Urological Pathology (ISUP) [20].

Statistical analysis

The data were expressed as absolute frequency and percentage and associated with recurrence within three

years using Fisher's exact test or Pearson's chi-square test (bivariate analysis) and multinomial logistic regression (multivariate analysis). All analyses were performed using SPSS v20.0 for Windows, with a confidence level of 95%.

Results

Clinical and pathological profile of prostate cancer patients

A total of 488 men were included in this study, with a mean age of 64.92±6.55 years, ranging from 42 to 80. Most patients (n=371, 76.0%) were over 60 years old. The mean preoperative PSA was 13.70±12.45 ng/ml, ranging from 1 to 133 ng/ml. Most patients had a preoperative PSA level of up to 10 ng/ml (n=278, 59.1%) (Table 1).

From a surgical perspective, 122 (25.0%) patients had clear margins, and 373 (76.4%) were staged as pT2, followed by pT3a (n=92, 18.9%) and pT3b (n=23, 4.7%). Only 13 (2.7%) patients had involved lymph nodes, and histologically, most patients had a Gleason score of 3+4 (n=205, 42.0%) or up to 3+3 (n=161, 33.0%) (Table 1).

No patient exhibited biochemical persistence after

initial treatment, and adjuvant therapy was not required. However, 117 (18.4%) patients experienced biochemical recurrence within three years. Salvage therapy was performed in 130 (26.6%) patients, and only five (1.0%) patients developed metastases during the evaluation period (Table 1).

Immunohistochemical profile of prostate cancer patients in the period

Following immunohistochemical evaluation, the mean immunohistochemical expression for *p16* was 37.38% ± 27.32%, ranging from 0 to 95% (Figure 1). Most cases showed an immunohistochemical expression percentage for *p16* of up to 50% of marked cells (n=294, 64.3%). The mean histoscore was 2.70 ± 2.24, ranging from 0 to 12. The most frequent histoscores were 1 (n=150, 32.8%) and 2 (n=117, 25.6%). Only 25 (5.5%) cases were entirely negative for *p16*, and 2 cases (0.4%) exhibited the maximum histoscore (Table 2, Figure 1).

Risk factors for biochemical recurrence in prostate cancer

However, patients with preoperative PSA >10 ng/ml (p=0.007), involved margins (p<0.001), pT3 staging (p<0.001), involved lymph nodes (p<0.001), and Gleason score higher than 3+4 exhibited a higher frequency of recurrence. Patients with up to 50% of immunopositive cells for *p16* (p=0.035) or low Histoscore (up to 3) (p=0.004) also showed a higher frequency of biochemical recurrence at three years (Table 3).

In multivariate analysis, Gleason score (>3+4) increased the risk of recurrence at three years by 3.08 times (95% CI = 1.69-5.62) (p<0.001), as well as involved margins which increased this risk by 2.93 times (95% CI = 1.70-5.04) (p<0.001), and *p16* with Histoscore up to 3 which increased it by 2.49 times (95% CI = 1.17-5.32) (p=0.018), independent of other variables (Table 4).

Discussion

In the present study, we sought to assess the

Table 1. Clinical and Pathological Profile of Patients with Prostate Cancer pT2-pT3b

	n	%
Age (64.92±6.55; 42-80 years old)		
Up to 60 years	117	24.0
>60 years	371	76.0
Preoperative PSA (13.70±12.45; 1-133)		
Up to 10 ng/ml	278	59.1
>10 ng/ml	192	40.9
Free margins		
No	366	75.0
Yes	122	25.0
Staging		
pT2	373	76.4
pT3a	92	18.9
pT3b	23	4.7
Lymph nodes		
Free	462	97.3
Involved	13	2.7
Gleason		
Minor equal 3	161	33.0
3+4	205	42.0
4+3	71	14.5
8 to 10	51	10.5
Biochemical persistence	0	0.0
Adjuvance	0	0.0
Biochemical recurrence in 3 years		
No	371	76.0
Yes	117	18.4
Rescue	130	26.6
Metastasis Development	5	1.0

Data expressed in the form of absolute frequency and percentage.

Table 2. Immunohistochemical Profile of Patients with Prostate Cancer pT2-PT3b

	n	%
p16 (37.38±27.32%; 0-95%)		
Up to 50%	294	64.3
>50%	163	35.7
p16 histoscore (2.70±2.24)		
0	25	5.5
1	150	32.8
2	117	25.6
3	50	10.9
4	45	9.8
6	35	7.7
8	32	7.0
9	1	0.2
12	2	0.4

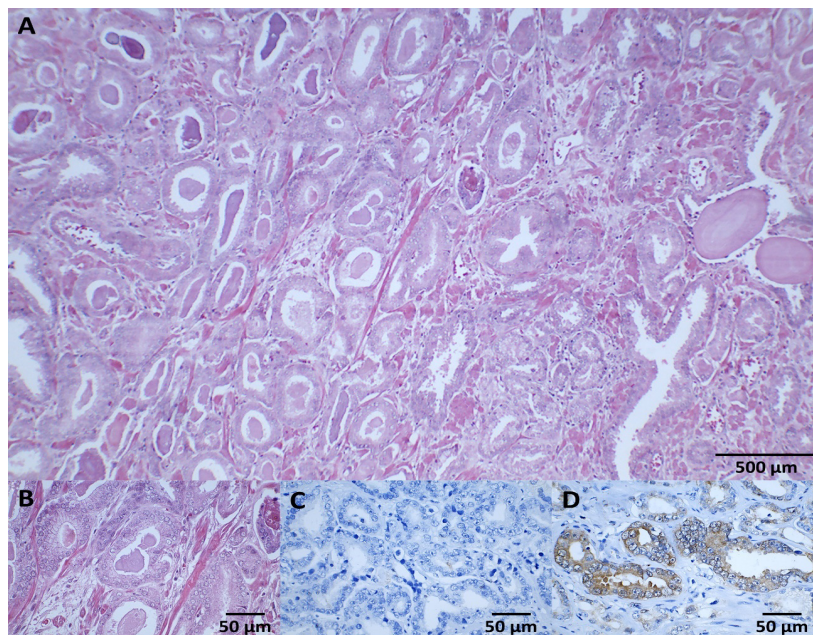Data expressed in the form of absolute frequency and percentage.

Figure 1. Histological and Immunohistochemical Profile for *p16* Expression in Prostate adenocarcinoma. (A) Microscopic appearance of prostate adenocarcinoma (H&E, 100x); (B) Microscopic appearance of prostate adenocarcinoma (H&E, 400x); (C) Negative immunohistochemical expression profile for *p16* in prostate adenocarcinoma (DAB-H, 400x); (D) Positive immunohistochemical expression profile for *p16* in prostate adenocarcinoma (DAB-H, 400x).

Table 3. Risk Factors for Recurrence at Three Years of Patients with Prostate Cancer pT2-pT3b

	Recurrence at 3 years		
	No	Yes	p-Value
Age			
Up to 60	86 (23.2%)	31 (26.5%)	0.464
>60	285 (76.8%)	86 (73.5%)	
Preoperative PSA			
Up to 10	223 (62.6%)*	55 (48.2%)	0.007
>10	133 (37.4%)	59 (51.8%)*	
Involved Margins			
No	300 (80.9%)*	66 (56.4%)	<0.001
Yes	71 (19.1%)	51 (43.6%)*	
Staging			
pT2	302 (81.4%)*	71 (60.7%)	<0.001
pT3a	60 (16.2%)	32 (27.4%)*	
pT3b	9 (2.4%)	14 (12.0%)*	
Lymph nodes			
Free	357 (99.2%)*	105 (91.3%)	<0.001
Involved	3 (0.8%)	10 (8.7%)*	
Gleason			
Minor equal 3+3	147 (39.6%)*	14 (12.0%)	<0.001
3+4	159 (42.9%)*	46 (39.3%)	
4+3	39 (10.5%)	32 (27.4%)*	
8 a 10	26 (7.0%)	25 (21.4%)*	
p16 %			
Up to 50%	212 (61.6%)	82 (72.6%)*	0.035
>50%	132 (38.4%)*	31 (27.4%)	
p16 histoscore			
Up to 3	246 (71.5%)	96 (85.0%)*	0.004
>3	98 (28.5%)*	17 (15.0%)	

* p<0.05, Fisher's exact test or Pearson's chi-square test (n, %).

Table 4. Multivariate Analysis of Predictors of Biochemical Recurrence at Three Years in Patients with Prostate Cancer pT2-pT3b

	p-Value	aHR (CI95%)
Recurrence at 3 Years		
Age (>60)	0.175	1.51 (0.83-2.73)
PSA (>10)	0.107	1.53 (0.91-2.58)
Gleason (>3+4)	*<0.001	3.08 (1.69-5.62)
Involved margins	*<0.001	2.93 (1.70-5.04)
Staging	0.207	2.13 (0.66-6.88)
Lymph nodes	0.117	4.20 (0.70-25.28)
p16 <50%	0.416	1.31 (0.69-2.49)
p16 (histoscore up to 3)	*0.018	2.49 (1.17-5.32)

* p<0.05, multinomial logistic regression; aHR, adjusted hazard ratio; 95% CI, 95% confidence interval of the aHR.

influence of *p16* immunohistochemical expression on the biochemical recurrence rate of prostate cancer. It was possible to observe a protective role of *p16* in these tumors. In addition, we identified independent prognostic factors for recurrence within three years, including *p16* immunohistochemical expression.

In the present sample, most cases were in patients over 60 years of age, data similar to the literature that shows that adenocarcinomas are more common from the fifth decade of life onwards, with an increase from that period onwards [21, 22]. Furthermore, 75% of the sample presented Gleason scores with a good prognosis, with scores 3+4 or 3+3 being the most frequent. It is known that Gleason scores are the most reliable clinical predictor of disease progression, with scores >7 having a higher risk of extraprostatic extension [23].

Several factors can influence recurrence; however, not

all men who experience biochemical recurrence progress in pathology, particularly at the end of initial treatment, where approximately 15 to 52% of patients experience disease recurrence, initially detected due to elevated serum PSA levels, known as biochemical recurrence [24, 25].

Although means for detecting PSA at deficient levels are already available, in patients who have undergone radical prostatectomy, biochemical recurrence typically occurs with serum PSA values above 0.2 ng/mL significant [24]. At the end of radiotherapy, a PSA value elevated by 2 ng/mL above the patient's NADIR is considered significant [24, 25]. In the studied sample, Gleason scores were higher than 3+4, and involvement of surgical margins was independently associated with biochemical recurrence.

After radical prostatectomy (RP), both metastasis and biochemical recurrence have prognostic implications. These are mainly classical pathological classifications such as extraprostatic extension (pT3a), seminal vesicle involvement (pT3b), and positive surgical margins, which are used to classify patients at high risk of biochemical recurrence [26, 27].

In this regard, loss of *p16* expression has been shown to have significant prognostic value in the biochemical recurrence of these tumors [28]. P16 is a regulatory protein that negatively regulates the cell cycle by inhibiting cyclin-dependent kinase 4 (CDK4) through interaction with CDK6 and cyclin D1 [28]. Increased levels of *p16* lead to decreased phosphorylation of the retinoblastoma protein (Rb), thereby inhibiting the progression of the cell cycle from the G1 to the S phase [6]. Inactivation of *p16* by various factors can promote or even be the causal factor of multiple neoplasms [28, 6].

In oropharyngeal and cervical tumors, positivity for *p16* is associated with a better prognosis [29, 30] Sethi et al., 2000a. Similarly, studies in the oral cavity suggest a protective role of *p16* in disease progression [31] and in various other tissues [29, 32]. Inoculation of adenovirus containing *p16* vectors drastically suppresses cell growth, leading prostate adenocarcinoma cell cultures to senescence [33] by suppressing pRB [34], thus making *p16* immunohistochemical expression in prostate adenocarcinomas a supportive diagnostic tool, given its direct relationship with Gleason scores [11].

Chakravarti et al. [12] and Kudahetti et al. [35] described a direct relationship between loss of expression of this protein and poor prognosis in prostate adenocarcinoma. On the other hand, Boldrini et al. [36] and Takahara et al. [37] showed that in advanced prostate adenocarcinomas, *p16* immunohistochemical expression is directly associated with higher Gleason scores, thus serving as a predictor of poor prognosis. Therefore, depending on the clinical and pathological stage, *p16* immunohistochemical expression exhibits distinct behaviors in prostate adenocarcinoma.

The main limitation of this study is that we only studied recurrence-free survival since most of these tumors have a low mortality rate, making it impossible to analyze overall survival. However, recurrence-free survival is one of the primary outcomes that impact overall survival in prostate cancer and is a critical analysis parameter considered in this study.

In Conclusion, the loss of *p16* immunohistochemical expression has been directly associated with a high rate of biochemical recurrence in prostate adenocarcinomas, serving as an independent predictor of poor prognosis. In addition, Gleason scores (>3+4) and compromised margins increase the risk of recurrence in an average of three years.

Author Contribution Statement

Acknowledgements

References

1. Inca, national cancer institute 2023. Estimate 2023: Cancer incidence in brazil. Inca. 2023.

2. Ferlay J, Ervik M, Lam F, Colombet M, Mery L, Piñeros M, et al. Global cancer observatory: Cancer today. Lyon: International agency for research on cancer. 2020 nov 25;20182020.

3. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209-49. https://doi.org/10.3322/caac.21660.

4. American cancer society. Cancer facts & figures 2019. American cancer society. 2019.

5. Maule M, Merletti F. Cancer transition and priorities for cancer control. Lancet Oncol. 2012;13(8):745-6. https://doi.org/10.1016/s1470-2045(12)70268-1.

6. LaPak KM, Burd CE. The molecular balancing act of *p16*(ink4a) in cancer and aging. Mol Cancer Res. 2014;12(2):167-83. https://doi.org/10.1158/1541-7786.Mcr-13-0350.

7. Tommasino M. The human papillomavirus family and its role in carcinogenesis. Semin Cancer Biol. 2014;26:13-21. https://doi.org/10.1016/j.semcancer.2013.11.002.

8. Brazil. Ministry of health. (org.). Clinical protocol and therapeutic guidelines for comprehensive care for people with sexually transmitted infections (stis). 2022.

9. Bogani G, Leone Roberti Maggiore U, Signorelli M, Martinelli F, Ditto A, Sabatucci I, et al. The role of human papillomavirus vaccines in cervical cancer: Prevention and treatment. Crit Rev Oncol Hematol. 2018;122:92-7. https://doi.org/10.1016/j.critrevonc.2017.12.017.

10. Nelson CW, Mirabello L. Human papillomavirus genomics: Understanding carcinogenicity. Tumour Virus Res.

2023;15:200258. https://doi.org/10.1016/j.tvr.2023.200258.

11. Remo A, Pancione M, Zanella C, Manfrin E. P16 expression in prostate cancer and nonmalignant lesions: Novel findings and review of the literature. Appl Immunohistochem Mol Morphol. 2016;24(3):201-6. https://doi.org/10.1097/pai.0000000000000171.

12. Chakravarti A, Heydon K, Wu CL, Hammond E, Pollack A, Roach M, et al. Loss of *p16* expression is of prognostic significance in locally advanced prostate cancer: An analysis from the radiation therapy oncology group protocol 86-10. J Clin Oncol. 2003;21(17):3328-34. https://doi.org/10.1200/jco.2003.12.151.

13. Magalhães IA, Costa GAJ, Borges MMF, Carlos A, Mesquita KC, Arruda LMA, et al. Deficiency in infiltration of cd8/cd3 positive lymphocytes and macrophages plays a role in lip carcinogenesis: An immunohistochemical study. Braz J Otorhinolaryngol. 2024;90(2):101379. https://doi.org/10.1016/j.bjorl.2023.101379.

14. Epstein JI, Egevad L, Amin MB, Delahunt B, Srigley JR, Humphrey PA. The 2014 international society of urological pathology (isup) consensus conference on gleason grading of prostatic carcinoma: Definition of grading patterns and proposal for a new grading system. Am J Surg Pathol. 2016;40(2):244-52. https://doi.org/10.1097/pas.0000000000000530.

15. Hirth CG, Vasconcelos GR, Lima MVA, da Cunha M, Frederico IKS, Dornelas CA. Prognostic value of fus immunoexpression for gleason patterns and prostatic adenocarcinoma progression. Ann Diagn Pathol. 2021;52:151729. https://doi.org/10.1016/j.anndiagpath.2021.151729.

16. Gonçalves AS, Mosconi C, Jaeger F, Wastowski IJ, Aguiar MCF, Silva TA, et al. Overexpression of immunomodulatory mediators in oral precancerous lesions. Hum Immunol. 2017;78(11-12):752-7. https://doi.org/10.1016/j.humimm.2017.09.003.

17. Eminaga O, Hinkelammert R, Titze U, Abbas M, Eltze E, Bettendorf O, et al. The presence of positive surgical margins in patients with organ-confined prostate cancer results in biochemical recurrence at a similar rate to that in patients with extracapsular extension and psa ≤ 10 ng/ml. Urol Oncol. 2014;32(1):32.e17-25. https://doi.org/10.1016/j.urolonc.2012.11.021.

18. Epstein JI, Oxley J, Ro JY, Van Der Kwast T, Zhou M. Tumours of the prostate: Intraductal carcinoma. Who classification of tumours of the urinary system and male genital organs. Lyon, france: International agency for research on cancer. 2016:164-5.

19. Holger M, Antonio Lc, Peter Ah, et al. Tumours of the prostate. In: World health organization classification of tumours of the urinary system and male genital organs. 4th. Ed. Iarc, 2016;135-83.

20. Tan PH, Cheng L, Srigley JR, Griffiths D, Humphrey PA, van der Kwast TH, et al. International society of urological pathology (isup) consensus conference on handling and staging of radical prostatectomy specimens. Working group 5: Surgical margins. Mod Pathol. 2011;24(1):48-57. https://doi.org/10.1038/modpathol.2010.155.

21. Rawla P. Epidemiology of prostate cancer. World J Oncol. 2019;10(2):63-89. https://doi.org/10.14740/wjon1191.

22. Tuppin P, Samson S, Fagot-Campagna A, Lukacs B, Alla F, Paccaud F, et al. Prostate cancer outcomes in france: Treatments, adverse effects and two-year mortality. BMC Urol. 2014;14:48. https://doi.org/10.1186/1471-2490-14-48.

23. Mikel Hubanks J, Boorjian SA, Frank I, Gettman MT, Houston Thompson R, Rangel LJ, et al. The presence of extracapsular extension is associated with an increased risk of death from prostate cancer after radical prostatectomy for patients with seminal vesicle invasion and negative lymph nodes. Urol Oncol. 2014;32(1):26.e1-7. https://doi.org/10.1016/j.urolonc.2012.09.002.

24. Cornford P, Bellmunt J, Bolla M, Briers E, De Santis M, Gross T, et al. Eau-estro-siog guidelines on prostate cancer. Part ii: Treatment of relapsing, metastatic, and castration-resistant prostate cancer. Eur Urol. 2017;71(4):630-42. https://doi.org/10.1016/j.eururo.2016.08.002.

25. Roach III M, Hanks G, Thames Jr H, Schellhammer P, Shipley WU, Sokol GH, et al. Defining biochemical failure following radiotherapy with or without hormonal therapy in men with clinically localized prostate cancer: Recommendations of the rtog-astro phoenix consensus conference. Int J Radiat Oncol Biol Phys. 2006;65(4):965-74. https://doi.org/10.1016/j.ijrobp.2006.04.029.

26. Thompson IM, Tangen CM, Paradelo J, Lucia MS, Miller G, Troyer D, et al. Adjuvant radiotherapy for pathological t3n0m0 prostate cancer significantly reduces risk of metastases and improves survival: Long-term followup of a randomized clinical trial. J Urol. 2009;181(3):956-62. https://doi.org/10.1016/j.juro.2008.11.032.

27. Wiegel T, Bottke D, Steiner U, Siegmann A, Golz R, Störkel S, et al. Phase iii postoperative adjuvant radiotherapy after radical prostatectomy compared with radical prostatectomy alone in pt3 prostate cancer with postoperative undetectable prostate-specific antigen: Aro 96-02/auo ap 09/95. J Clin Oncol. 2009;27(18):2924-30. https://doi.org/10.1200/jco.2008.18.9563.

28. Sallum LF, Andrade L, Ramalho S, Ferracini AC, de Andrade Natal R, Brito ABC, et al. Wt1, p53 and *p16* expression in the diagnosis of low- and high-grade serous ovarian carcinomas and their relation to prognosis. Oncotarget. 2018;9(22):15818-27. https://doi.org/10.18632/oncotarget.24530.

29. Salazar CR, Anayannis N, Smith RV, Wang Y, Haigentz M, Jr., Garg M, et al. Combined *p16* and human papillomavirus testing predicts head and neck cancer survival. Int J Cancer. 2014;135(10):2404-12. https://doi.org/10.1002/ijc.28876.

30. Fonseca fv, tomasich fds, jung je, et al. The role of immunohistochemical expression of *p16*ink4a and p53 in predicting recurrence of cin-ag after conization treatment. Rev col bras cir. 2016;43:035-041.

31. Ang KK, Sturgis EM. Human papillomavirus as a marker of the natural history and response to therapy of head and neck squamous cell carcinoma. Semin Radiat Oncol. 2012;22(2):128-42. https://doi.org/10.1016/j.semradonc.2011.12.004.

32. Sethi S, Ali-Fehmi R, Franceschi S, Struijk L, van Doorn LJ, Quint W, et al. Characteristics and survival of head and neck cancer by hpv status: A cancer registry-based study. Int J Cancer. 2012;131(5):1179-86. https://doi.org/10.1002/ijc.26500.

33. Steiner MS, Zhang Y, Farooq F, Lerner J, Wang Y, Lu Y. Adenoviral vector containing wild-type *p16* suppresses prostate cancer growth and prolongs survival by inducing cell senescence. Cancer Gene Ther. 2000;7(3):360-72. https://doi.org/10.1038/sj.cgt.7700151.

34. Steiner MS, Wang Y, Zhang Y, Zhang X, Lu Y. P16/mts1/ink4a suppresses prostate cancer by both prb dependent and independent pathways. Oncogene. 2000;19(10):1297-306. https://doi.org/10.1038/sj.onc.1203428.

35. Kudahetti SC, Fisher G, Ambroisine L, Prowse D, Kattan MW, Foster CS, et al. Immunohistochemistry for *p16*, but not rb or p21, is an independent predictor of prognosis in conservatively treated, clinically localised prostate cancer. Pathology. 2010;42(6):519-23. https://doi.org/10.3109/00313025.2010.508788.

36. Boldrini L, Manassero F, Selli C, Bartoletti R, Faviana P. P16 and its putative interplay with metabolic factors in prostate cancer: An analysis based on public tcga data. World Acad Sci J. 2020. https://doi.org/10.3892/wasj.2020.33.

37. Takahara T, Satou A, Sugie M, Watanabe M, Kanao K, Sumitomo M, et al. Prognostic significance of *p16* expression in high-grade prostate adenocarcinoma. Pathol Int. 2020;70(10):743-51. https://doi.org/10.1111/pin.12997.