ORIGINAL PAPER

RJME Romanian Journal of Morphology & Embryology http://www.rjme.ro/

# Immunoexpression of Cyclin D1, P53 and Ki67 in prostatic acinar adenocarcinomas

ADRIAN MIHAI STOICULESCU[1)], OANA IULIA CREŢU[2)], BIANCA CĂTĂLINA ANDREIANA[2)], DAN ŞTEFAN DIACONESCU[3)], PETRU OCTAVIAN DRĂGOESCU[4)], ALEX EMILIAN STEPAN[2)]

[1)]*PhD Student, Department of Pathology, University of Medicine and Pharmacy of Craiova, Romania*

[2)]*Department of Pathology, University of Medicine and Pharmacy of Craiova, Romania*

[3)]*Clinic of Urology I, Prof. Dr. Theodor Burghele Clinical Hospital, Bucharest, Romania*

[4)]*Department of Urology, University of Medicine and Pharmacy of Craiova, Romania*

## Abstract

Prostate cancer continues to be a health problem by epidemiological indicators, despite prevention and detection programs and advanced knowledge of molecular biology. Acinar forms are the most common and constitute a tumor group with a relatively good prognosis, but sometimes unpredictable compared to the associated histopathological (HP) parameters. Among the molecular mechanisms involved in tumor cell proliferation is the disruption of the cell cycle, and the evaluation of the expression of key proteins involved can assist the histological stratification of cases. The study investigated the immunoexpression of Cyclin D1, P53 and Ki67 in 55 prostatic acinar adenocarcinomas (PAAs), in relation to the HP prognostic parameters of the lesions. High reaction scores expressed as positivity index (PI) indicated the significant association of the three markers with increased grading groups recommended by *International Society of Urological Pathology* (ISUP), perineural and lymphovascular invasion. P53 PI and Ki67 PI were significantly or at the limit of significance increased in conventional and colloidal PAA, and in the case of P53 and in addition in advanced stages. Analysis of the effective values of the reactions indicated significant positive linear correlations between the investigated immunomarkers. The reactions were variable in a relatively homogeneous group of PAA and although they were generally associated with aggressive HP behavior, they seem useful in the punctual identification of cases that require a particular management, in the context of specific oncological therapy.

***Keywords***: prostatic adenocarcinoma, Cyclin D1, P53, Ki67.

## Introduction

Prostatic acinar adenocarcinomas (PAA) are the most common age-dependent malignant tumors of men, with variable incidence and an increasing mortality rate due to advanced disease, ranking them after lung cancer [1, 2]. Although they generally have a better prognosis compared to other malignant tumors and can be easily diagnosed through screening programs, there are many cases that deviate from the classic biological behavior associated with tumor grade and that contribute to worsening epidemiological data [3]. Identification of useful markers for refining the criteria for assessing PAA with evolutionary potential may improve the prognosis of patients. The relationship of tumor cell proliferation with cell cycle disruption is generally well known in the progression of malignant lesions, which seem to depend on the alteration of signaling pathways and checkpoints in the form of non-lethal modifications that allow tumor cell survival [4].

Cyclin D1 is an essential regulator of the cell cycle, with a role in cell proliferation and in the mechanisms of resistance to hormonal therapy of PAA, with controversial data regarding the level of expression in relation to the aggressiveness of prostate tumors [5–7]. At the same time, one of the guardians of the cell cycle represented by p53, with a role in the control of progression in the cycle and in apoptosis, is identified in many studies as a marker of advanced PAA with a reserved prognosis [8, 9], although there are studies that do not support the association with some histological parameters of aggressiveness of the lesions [10, 11]. The variability of the reported results may be due to the heterogeneity of prostate carcinomas, which in addition to different growth patterns, can present neuro-endocrine or sarcomatoid differentiations present in a particular stromal microenvironment and dependent on endocrine sensitivity and interaction with paracrine signaling [12–14].

In the context in which the two proteins have an essential role in cell proliferation both directly and through complex interactions with other biomolecular mechanisms of progression, it is of interest to know the expression in PAA and the potential for their therapeutic interference. Also, the hormonal dependence of PAA and the interaction of Cyclin D1 with the status of androgen receptors (ARs) seems to be one of the mechanisms that stimulate tumor proliferation [15]. This aspect is also supported by the diminution to the loss of Cyclin D1 immunoexpression in hormone-independent nonacinar prostate carcinomas [16]. Moreover, some authors have supported significant associations of cyclin with estrogen receptor beta (ER$\beta$)

immunoreactivity suggesting a possible estrogen-dependent regulation of this protein [17].

Most studies designate Ki67 as a good indicator of tumor proliferation rate in PAA and of aggressiveness and prognosis, being generally consistent with the histological parameters of tumor lesions [18, 19].

### Aim

The study followed the immunoexpression of Cyclin D1, p53 and Ki67 in relation to the main histological prognostic parameters of PAAs.

## Materials and Methods

In this study, we analyzed 55 PAAs, which were diagnosed within the Department of Pathology, Emergency County Hospital, Craiova, Romania, during 2020–2023, in patients operated on in the Department of Urology of the same Hospital. The study included only primitive PAA, with no history of previous oncological treatments, no history of local treatments or repeated endoscopies for benign nodular hyperplasia or acute chronic inflammation. The biological material was represented by prostatectomy specimens or transurethral resection of the prostate (TURP) fragments, without major electrocoagulation artifacts and without primary processing defects. If a case had TURP and prostatectomy fragments available, the resection fragments were chosen if pre-prostatectomy oncological treatments were performed or prostatectomy if radical surgery was performed directly. No biopsy fragments were selected, and cases with associated prostatectomy were selected under the same conditions as previously described. Classification and assessment of PAA was performed in accordance with the latest *World Health Organization* (WHO) criteria for PAA [20].

For histopathological (HP) analysis, the biological material was processed by paraffin embedding and staining with Hematoxylin–Eosin (HE), and tumor subtype, grading groups recommended by *International Society of Urological Pathology* (ISUP), lymphovascular invasion (LVI), perineural invasion (PNI) and tumor stage were followed. The distribution of cases in relation to HP parameters was analyzed in relation to the immunoexpression of Cyclin D1 (rabbit monoclonal antibody), P53 and Ki67 (mouse monoclonal antibodies) (Table 1).

**Table 1 – *Protocols for IHC reactions***

| Antibody | Clone/ Manufacturer | Dilution | Pretreatment (HIER) | External positive control |
|---|---|---|---|---|
| Cyclin D1 | EP12/Agilent Dako | 1:100 | Tris/EDTA buffer, pH 9 | Tonsil |
| P53 | DO-7/Agilent Dako | 1:50 | Tris/EDTA buffer, pH 9 | OSCC |
| Ki67 | MIB-1/Agilent Dako | 1:75 | Citrate buffer, pH 6 | Tonsil |

EDTA: Ethylenediaminetetraacetic acid; HIER: Heat-induced epitope retrieval; IHC: Immunohistochemical; OSCC: Oral squamous cell carcinoma.

From the paraffin blocks, 4 μm sections were obtained on which immunohistochemical (IHC) reactions were performed and which were prepared for incubation with antibodies by deparaffinization with xylene, hydration with alcoholic solutions, washing in distilled water, antigen retrieval (Table 1), inhibition of endogenous peroxidase and non-specific sites with hydrogen peroxide and bovine serum albumin (BSA) 1.5%. After incubation with specific antibodies overnight at 4ºC, the sections were washed with phosphate-buffered saline (PBS) and incubated with the secondary antibody (20 minutes) from the EnVision™ FLEX+ System, which indicated the signals through polymeric amplification. These were visualized using the 3,3'-Diaminobenzidine (DAB) tetrahydrochloride chromogen, the reactions being interrupted after five minutes, followed by washing in PBS, running water and staining with Hematoxylin. Reactions were present at the level of the external positive controls used.

Semi-quantitative quantification of the IHC reactions obtained was performed by establishing a positivity index (PI) by reporting the number of labeled tumor cells to the total number of cells at 200× microscopic magnification, expressed as a percentage. For each case, five microscopic fields with maximum reactions were analyzed and the effective mean value of the reactions was established. For statistical analysis, in the case of Cyclin D1, low PI (<25%), medium PI (25–50%) and high PI (>50%) were considered, in the case of P53, low PI (1–5%) and high PI (>5%), and for Ki67 low PI (1–5%), moderate PI (6–10%) and high PI (>10%). In this study, only the presence of the labels was evaluated, not their intensity, which was assessed only descriptively.

Images for evaluation and quantification were acquired by two pathologists using the KoPa Pro software camera attached to the Nikon Eclipse Ei-R microscope, and in case of incongruence of the results, they repeated the counting until consensus was reached.

For statistical analysis, means, standard deviations and comparison tests [$\chi^2$ (*chi*-squared), Fisher, Pearson] were performed from the Statistical Package for the Social Sciences (SPSS) 12, differences or correlations being significant or at the limit for $p<0.05$ and $p<0.1$, respectively. In this study, negative PIs were considered for statistical analysis.

The study respected the informed consent of the patients and the norms of research ethics, being approved by the Local Ethics Committee (Approval No. 223/28.09.2023).

## Results

The investigated tumors were represented by PAAs, diagnosed in a group of patients with a mean age of 70.1±11.6 years, most of which were of the conventional type (78.2%), classified in ISUP 2 and 4 grading groups (47.2%), with PNI in almost half of the cases (49.1%), consistent LVI (18.2%), and which most often presented extracapsular extension (stage III – 48.5%) in cases with prostatectomy in which tumor staging was performed (35 cases) (Table 2).

**Table 2 – *Distribution of cases according to HP parameters***

| HP parameters | No. of cases |
|---|---|
| Histological type | CPAA: 43 |
| | FPAA: 5 |
| | APAA: 3 |
| | PPAA: 2 |
| | CoPAA: 2 |

| HP parameters | No. of cases |
|---|---|
| Grade groups (ISUP) | ISUP 1: 11 |
| | ISUP 2: 13 |
| | ISUP 3: 8 |
| | ISUP 4: 13 |
| | ISUP 5: 10 |
| PNI | Present: 27 |
| | Absent: 28 |
| LVI | Present: 10 |
| | Absent: 45 |
| Tumor stage | I: 3 |
| | II: 12 |
| | III: 17 |
| | IV: 3 |

APAA: Atrophic prostate acinar adenocarcinoma; CoPAA: Colloid prostate acinar adenocarcinoma; CPAA: Conventional prostate acinar adenocarcinoma; FPAA: Foamy prostate acinar adenocarcinoma; HP: Histopathological; ISUP: *International Society of Urological Pathology*; LVI: Lymphovascular invasion; PNI: Perineural invasion; PPAA: Pseudohyperplastic prostate acinar adenocarcinoma.

Cyclin D1 immunoexpression was present at the nuclear level in 94.5% of the PAA investigated, for the entire analyzed group the effective mean PI value was 40±21.8, the reaction values varying within quite wide limits. The reactions were also present in normal glandular and ductal areas, in those with benign nodular hyperplasia and other associated benign changes (atrophy, adenosis, basal hyperplasia) in 5–10% of the cells, generally with weaker intensity than in the tumor areas. Some stromal cells (fibroblasts) showed nuclear staining.

Cyclin D1 reactions at the tumor level showed differences depending on the HP parameters analyzed, although the ranges of positive cells varied within the same tumor category. In relation to the HP type, the most important Cyclin D1 reactions were present in colloid PAA (CoPAA), conventional PAA (CPAA) and foamy PAA (FPAA), with mean PI values of 45, 44.4±20.1 and 32±23.6, compared to atrophic PAA (APAA) and pseudohyperplastic PAA (PPAA), with values of 8.3±2.8 and 7.5, respectively (Table 3) (Figure 1, A–D). ISUP 4 and 5 groups presented the highest PI values, 48±12.6 and 54.5±23.6, followed in order by ISUP 3, 2 and 1 groups, with values of 44.3±23.2, 41.1±16.2 and 12.7±8.1, respectively (Table 3). PAA with PNI and vascular invasion had mean Cyclin D1 PI values of 48.8±20.1 and 54.5±23.6, while tumors without this type of invasion had mean PI values of 32.2±18.9 and 36.7±20.2, respectively (Table 3). PAA in stages III/IV had reaction values of 51.1±19 and 63.3±20.8, compared to stages I/II, with values of 8.3±7.6 and 37.9±17.2, respectively (Table 3).

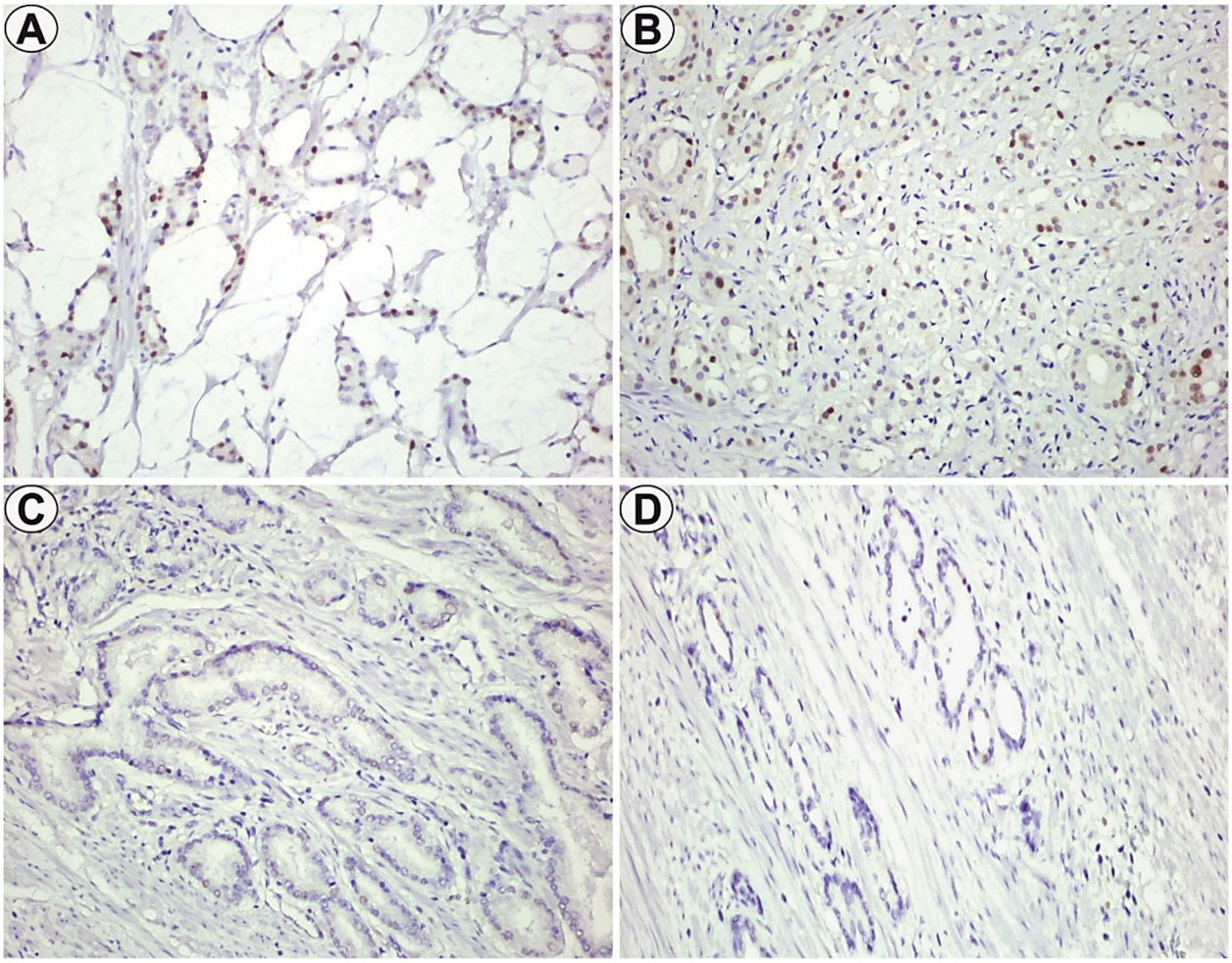


**Figure 1** – ***Prostate acinar adenocarcinoma, Cyclin D1 immunostaining, ×200: (A) CoPAA type; (B) CPAA type; (C) PPAA type; (D) APAA type. APAA: Atrophic prostate acinar adenocarcinoma; CoPAA: Colloid prostate acinar adenocarcinoma; CPAA: Conventional prostate acinar adenocarcinoma; PPAA: Pseudohyperplastic prostate acinar adenocarcinoma.***

**Table 3 –** ***Distribution of positivity intervals for the analyzed markers***

| HP parameters/*p*-value | | Cyclin D1 (range of positive cells) | P53 (range of positive cells) | Ki67 (range of positive cells) |
|---|---|---|---|---|
| Histological type | CPAA | 15–80 | 1–50 | 1–25 |
| | FPAA | 15–70 | 1–2 | 1–5 |
| | APAA | 5–10 | 5 | 1 |
| | PPAA | 5–10 | Negative | 1 |
| | CoPAA | 40–50 | 5–35 | 25–50 |
| | *p*-value ($\chi^2$ test) | <0.001 | 0.345 | 0.071 |
| Grade groups (ISUP) | ISUP 1 | 5–25 | 1–5 | 1–7 |
| | ISUP 2 | 15–75 | 1–4 | 1–20 |
| | ISUP 3 | 30–70 | 1–5 | 1–50 |
| | ISUP 4 | 30–70 | 1–5 | 1–25 |
| | ISUP 5 | 40–80 | 1–50 | 1–25 |
| | *p*-value ($\chi^2$ test) | <0.001 | 0.001 | <0.001 |
| PNI | Present | 10–75 | 1–50 | 1–50 |
| | Absent | 5–70 | 1–35 | 1–25 |
| | *p*-value (Fisher's test) | 0.018 | 0.070 | 0.004 |
| LVI | Present | 40–80 | 3–50 | 1–25 |
| | Absent | 5–75 | 1–35 | 1–50 |
| | *p*-value (Fisher's test) | 0.039 | 0.003 | 0.003 |
| Tumor stage | I | 10–15 | Negative | 1–5 |
| | II | 20–70 | 1–5 | 1–50 |
| | III | 30–75 | 1–50 | 1–25 |
| | IV | 40–80 | 1–10 | 1–20 |
| | *p*-value ($\chi^2$ test) | 0.002 | 0.315 | 0.246 |

APAA: Atrophic prostate acinar adenocarcinoma; CoPAA: Colloid prostate acinar adenocarcinoma; CPAA: Conventional prostate acinar adenocarcinoma; FPAA: Foamy prostate acinar adenocarcinoma; HP: Histopathological; ISUP: *International Society of Urological Pathology*; LVI: Lymphovascular invasion; PNI: Perineural invasion; PPAA: Pseudohyperplastic prostate acinar adenocarcinoma.

Statistical analysis indicated significantly higher differences in CoPAA and CPAA ($p<0.001$, $\chi^2$ test), in ISUP 2–5 groups ($p<0.001$, $\chi^2$ test), which presented PNI ($p=0.018$, Fisher's test) and LVI ($p=0.039$, Fisher's test), and were in advanced stages ($p=0.002$, $\chi^2$ test) (Figure 2, A–D).

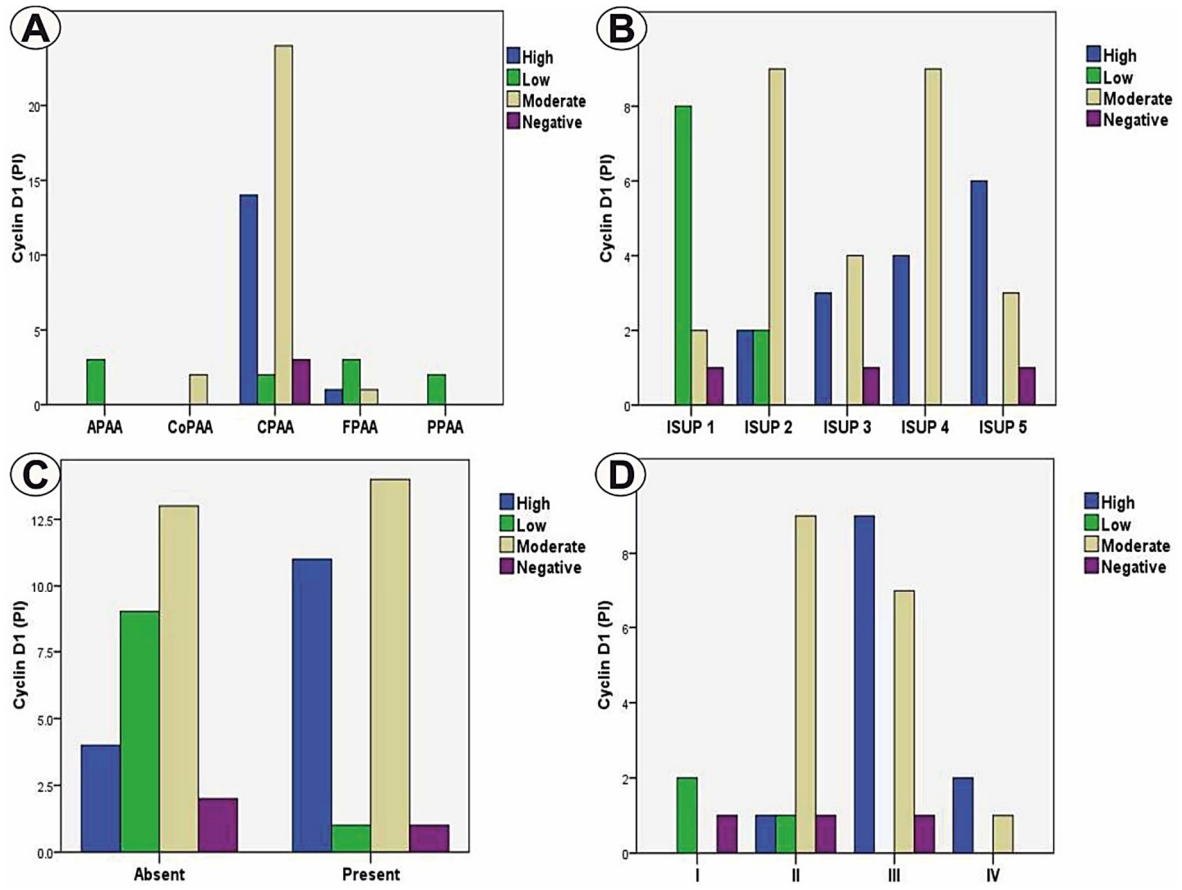


**Figure 2 –** ***Cyclin PI values distribution depending on tumor type (A), ISUP groups (B), PNI (C) and tumor stage (D). APAA: Atrophic prostate acinar adenocarcinoma; CoPAA: Colloid prostate acinar adenocarcinoma; CPAA: Conventional prostate acinar adenocarcinoma; FPAA: Foamy prostate acinar adenocarcinoma; ISUP: International Society of Urological Pathology; PI: Positivity index; PNI: Perineural invasion; PPAA: Pseudohyperplastic prostate acinar adenocarcinoma.***

P53 immunoreactions were present at the tumor level, in 49.1% of cases, at the nuclear level. We did not find staining at the non-tumor areas or at the stromal level. For the entire PAA group investigated, the average PI was

3±8.3. In general, the reactions were weaker compared to the other markers analyzed.

The highest reactions were present in CoPAA, with a mean PI of 20, followed by CPAA, APAA and FPAA, with values of 2.7±7.9, 1.6±2.8 and 0.6±0.8, respectively, while PPAA were negative (Table 3) (Figure 3, A–D). ISUP 5 was the only group with consistent P53 PI, with a value of 11.4±17.5, followed in order by ISUP 4, 3, 2 and 1 groups, with mean PI values of 1.6±2.3, 1.5±1.7, 1±1.1 and 0.5±1.5, respectively (Table 3). PAA with PNI and vascular invasion had PI values of 5.5±11.4 and 7.8±15.7, compared with those without these invasive features, with values of 2±6.1 and 1.9±5.3, respectively (Table 3). PAA in stages III and IV had P53 PIs of 7±14 and 3.6±5.5, compared with those in stage II, with PIs of 1.5±2.1, or those in stage I that were negative (Table 3).

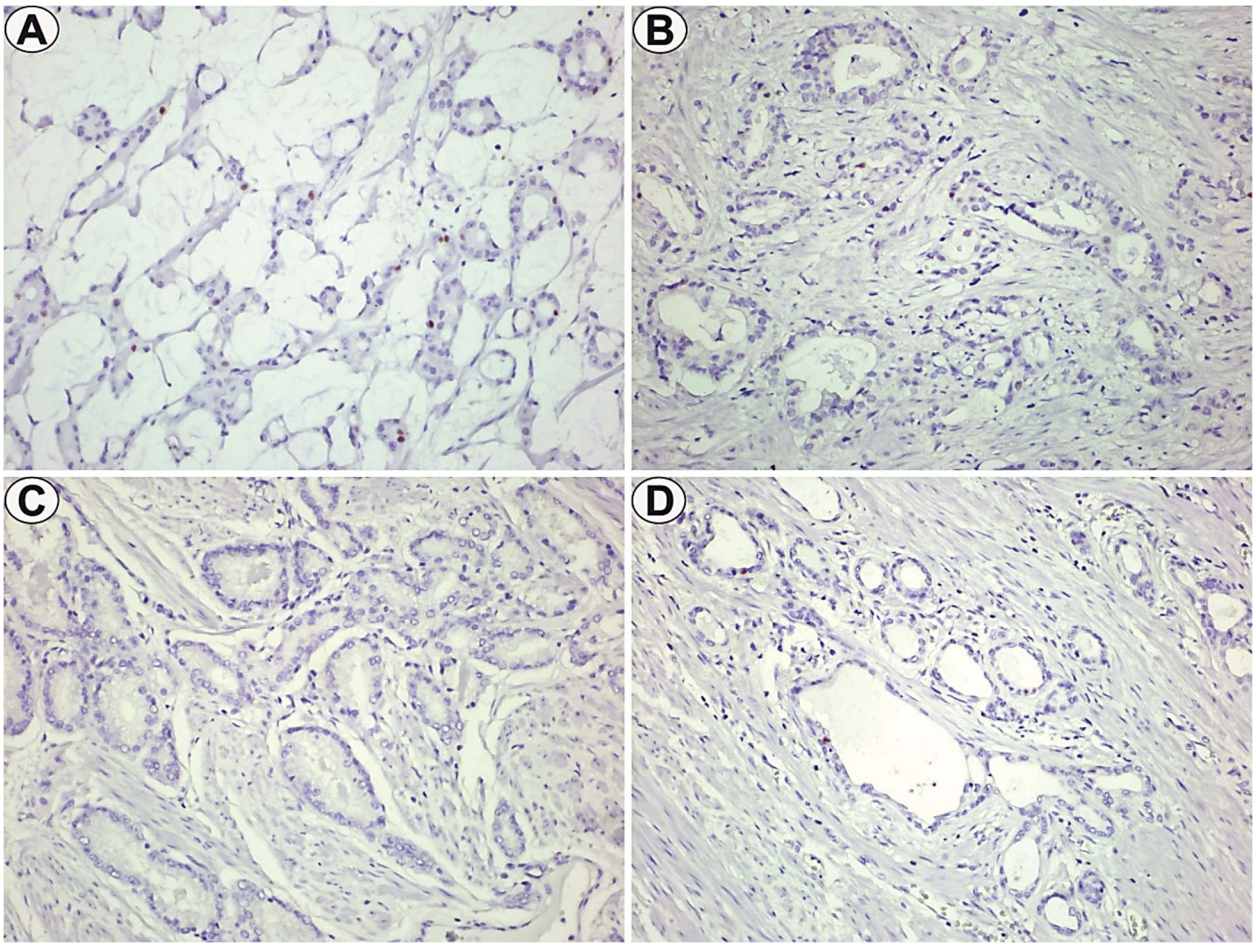


**Figure 3** – ***Prostate acinar adenocarcinoma, P53 immunostaining, ×200: (A) CoPAA type; (B) CPAA type; (C) PPAA type; (D) APAA type. APAA: Atrophic prostate acinar adenocarcinoma; CoPAA: Colloid prostate acinar adenocarcinoma; CPAA: Conventional prostate acinar adenocarcinoma; PPAA: Pseudohyperplastic prostate acinar adenocarcinoma.***

Statistical analysis of P53 reactions indicated significantly higher differences in PAA in the ISUP 5 group ($p$=0.001, $\chi^2$ test) and those with LVI ($p$=0.003, Fisher's test) (Table 3) (Figure 4, A and B). There were higher differences at the limit of statistical significance in the case of PAA with PNI ($p$=0.07, Fisher's test) (Table 3) (Figure 4C). There were no differences in P53 PI in relation to tumor type or tumor stage ($p$>0.1, $\chi^2$ test) (Table 3) (Figure 4D).

Ki67 immunoexpression was present in all PAA at the nuclear level, as well as in rare tumoral and epithelial stromal elements in non-neoplastic areas. For the analyzed group, the mean Ki67 PI value was 91±9.6.

The highest Ki67 PI values were identified in CoPAA, 37.5, followed by CPAA with PI of 9.4±7.6, FPAA with PI of 2.6±2.1 and APAA and PPAA with values of 1 (Table 3) (Figure 5, A–D). ISUP 3–5 groups had the most important reactions, with PI of 10.3±16.4, 13.1±7.9 and 16.1±7.5, compared to ISUP 1–2 groups, with values of 2.2±2.2 and 4.6±5.3, respectively (Table 3). PAA with PNI and vascular invasion had Ki67 PI of 12.9±10.5 and 14.1±6.7, compared to those that did not present such associations, respectively 6.9±7.8 and 8±9.8, respectively (Table 3). Regarding tumor stage, PAA in stages II/III had PI values of 13.9±12.9 and 12.5±8.7, compared to stages I/IV, with values of 2.3±2.3 and 8.6±10, respectively (Table 3).

Statistical analysis of Ki67 PI values indicated higher values, at the limit of significance for CoPAA ($p$=0.071, $\chi^2$ test) and significantly higher values in the case of PAA in ISUP 3–5 groups ($p$<0.001, $\chi^2$ test), with PNI ($p$=0.004, Fisher's test) and LVI ($p$=0.003, Fisher's test) (Figure 6, A–C). We did not find differences in P53 PI in relation to tumor stage ($p$=0.246, $\chi^2$ test) (Table 3).

Analysis of the distribution of effective values of Cyclin D1, P53 and Ki67 indicated statistically significant positive linear correlations between Cyclin D1/P53 ($p$=0.022, Pearson's test), Cyclin D1/Ki67 ($p$=0.010, Pearson's test) and P53/Ki67 ($p$=0.007, Pearson's test) (Figure 6D).

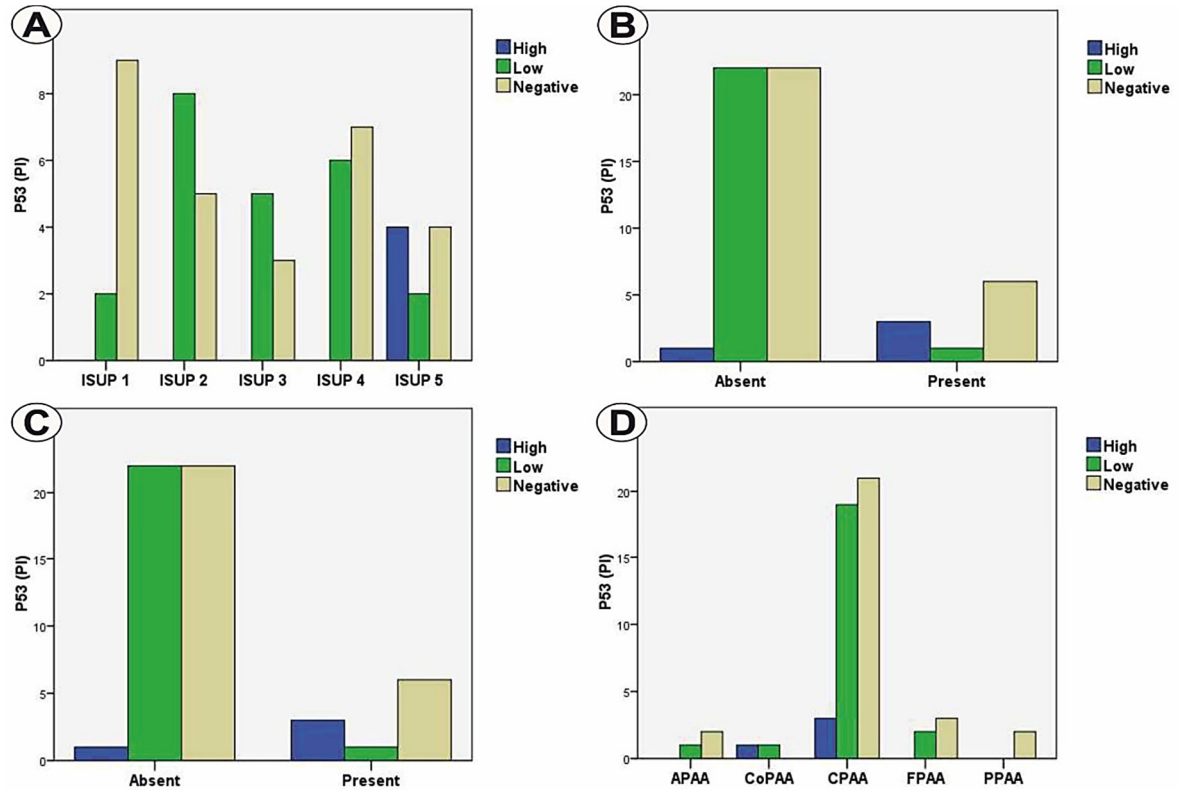


**Figure 4 –** ***P53 PI values distribution depending on ISUP groups (A), LVI (B), PNI (C) and tumor type (D). APAA: Atrophic prostate acinar adenocarcinoma; CoPAA: Colloid prostate acinar adenocarcinoma; CPAA: Conventional prostate acinar adenocarcinoma; FPAA: Foamy prostate acinar adenocarcinoma; ISUP: International Society of Urological Pathology; LVI: Lymphovascular invasion; PI: Positivity index; PNI: Perineural invasion; PPAA: Pseudohyperplastic prostate acinar adenocarcinoma.***

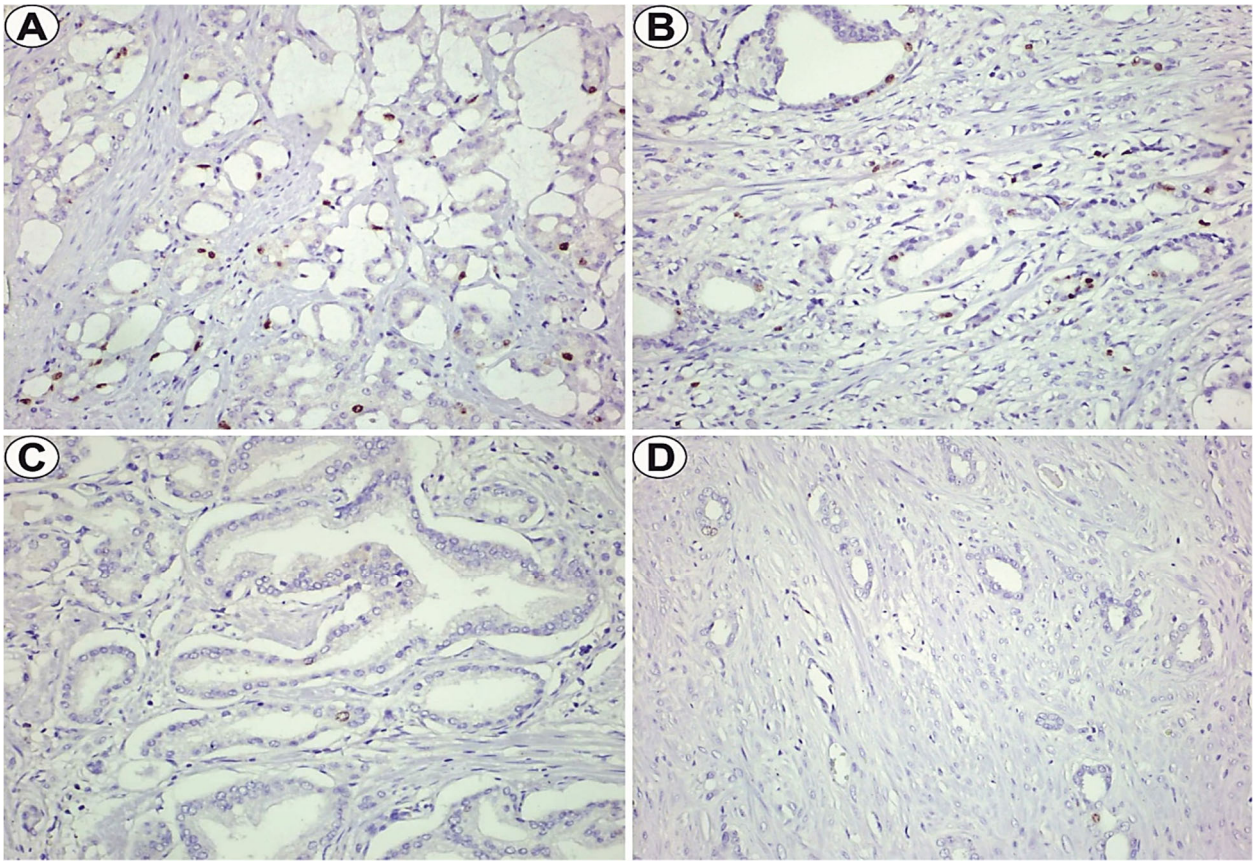


**Figure 5 –** ***Prostate acinar adenocarcinoma, Ki67 immunostaining, ×200: (A) CoPAA type; (B) CPAA type; (C) PPAA type; D) APAA type. APAA: Atrophic prostate acinar adenocarcinoma; CoPAA: Colloid prostate acinar adenocarcinoma; CPAA: Conventional prostate acinar adenocarcinoma; PPAA: Pseudohyperplastic prostate acinar adenocarcinoma.***

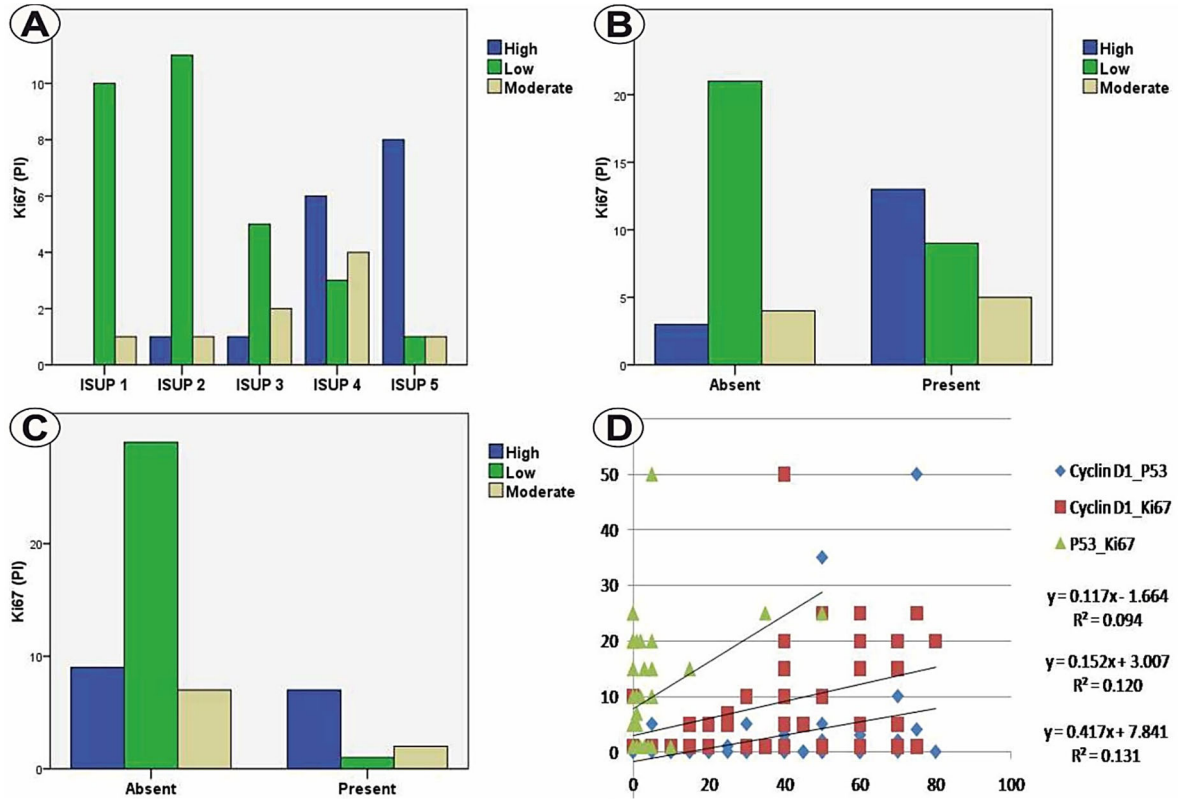


**Figure 6** – ***Ki67 PI values distribution depending on ISUP groups (A), PNI (B), LVI (C) and Cyclin D1, P53 and Ki67 distribution values (D). ISUP: International Society of Urological Pathology; LVI: Lymphovascular invasion; PI: Positivity index; PNI: Perineural invasion.***

## Discussions

As in the case of other malignant tumors, PAA is the result of multiple molecular mechanisms that interact and determine the appearance and evolution of cancer cells, including alteration of the epithelial phenotype and the intercellular adhesion system, angiogenesis and lymph-angiogenesis, acquisition of the mesenchymal phenotype, dysregulation of the cell cycle and apoptosis – all of these are achieved through signaling pathways dependent on numerous proteins with roles as growth factors, transcription factors, proteins involved in normal or altered metabolism, activated through autocrine or paracrine mechanisms [21–24].

Cyclin D1 and P53 are representative of the cell cycle. Mitogenic, hormonal and growth factors regulate the level of Cyclin D1 expression, which ensures the transition between the G1 and S phases by forming complexes with cyclin-dependent kinases 4/6 (CDK4/6) and phosphorylation of the retinoblastoma protein [25]. In PAA, the role of Cyclin D1 is complex, being influenced by the AR pathway, and the overexpression of this protein has been correlated with tumor progression and the emergence of resistance to antiandrogen therapies [15]. In the case of castration-resistant PAA, one of the factors involved is the aberrant activation of AR and androgen-independent cell proliferation, associated with the presence of the Cyclin D1b isoform [26]. Relatively recent studies indicate the therapeutic target value of the molecule, with blocking agents of the Cyclin D1/CDK4/6 complexes arresting the cell cycle in the G1 phase [5].

In our study, Cyclin D1 reactions were observed in the majority of PAAs (94.5%), as well as in non-tumor areas but with differences in staining, aspects consistent with other studies [27]. Cyclin D1 expression has been described in 70–95% of PAAs, with utility in differentiating malignant and benign prostatic lesions [17, 28, 29].

In this study, Cyclin D1 reactions showed significant differences in relation to tumor type, ISUP, PNI, LVI groups and tumor stage, higher values being associated with aggressive parameters of the lesions.

Data from the literature indicate both low expression and overexpression in PAA. Thus, Yu *et al.* observed that Cyclin D1 expression is higher in tumors with Gleason score ≥7 compared to those with lower scores, and reported a trend of correlation with PNI, without significant associations with prostate-specific antigen (PSA) levels [30]. In another study, Aaltomaa *et al.* reported a significant association between increased Cyclin D1 expression and PNI [31], and Mohammed *et al.* found associations with both PNI and PSA values [28]. Other studies have shown that Cyclin D1-positive prostate tumor cells exhibit higher motility, increased invasive capacity, and a hormone-independent phenotype in cell cultures [32]. However, other studies have not identified a clear correlation between Cyclin D1 expression and Gleason score [6, 7]. The study conducted by Drobnjak *et al.* on 86 primary tumors and 22 bone metastases showed that positive expression of Cyclin D1 was much more frequent in metastases compared to primary tumors, and correlations were also obtained with Gleason score and PSA level [6]. In recent studies, the authors obtained a significant correlation between Cyclin D1 expression and Gleason score, tumor stage, tumor size and treatment failure, but also with PNI and PSA values [28].

P53 immunoexpression has been extensively studied in the literature in prostate tumors, suggesting a frequency of tumor protein P53 (*TP53*) mutations of 10–20% in prostate adenocarcinomas treated by radical prostatectomy, with a tendency to increase to 30% in tumors with high Gleason grade [33]. *TP53* mutations are 3–5 times more frequent in advanced prostate cancer compared to tumors in early stages, which supports the idea that p53 expression is a relatively late event, associated with tumor progression [34, 35]. In a 2025 study, Ofner *et al.* emphasize that *TP53* deficiency consistently correlates with shorter time to relapse, higher distant metastasis rate and reduced overall survival (OS) [9]. Recent meta-analyses, which included both immunohistochemistry and sequencing studies, confirm that p53 overexpression is associated with decreased OS and a higher probability of relapse [35].

In our study, we found a relatively low positivity rate of PAA (49.1%), as well as effective values of P53 PI were low compared to Cyclin D1 and Ki67; however, high P53 PI was associated with high-grade, PNI and LVI. Although some studies have found a relationship between P53 overexpression and aggressiveness of prostate carcinomas [36–38], these appear to be the result of missense mutations with protein accumulation, as opposed to nonsense mutations and deletions resulting in loss of protein expression [39]. As such, aberrant P53 expression, which may relate to both circumstances, is discussed and the authors associate the overall low frequency of p53 alterations in clinically localized prostate cancer [40]. However, the survival rate of P53-positive prostate carcinomas is lower compared to negative ones [41], and the presence of P53 reactions is associated with advanced stage, high grade and early recurrence [40]. In this context, Kallakury *et al.* showed an association between nuclear p53 expression and lesions with high Gleason grade, as well as with extraprostatic invasion, suggesting that nuclear p53 accumulation is an indicator of tumor aggressiveness [42]. Subsequent studies, including those conducted by the same group, confirmed a significant correlation between p53 overexpression and tumors with high Gleason score [37, 43]. Another recent clinicopathological study, which compared p53 expression in benign hyperplasia, high-grade intraepithelial neoplasia, and prostatic carcinoma, showed that p53 increases from benign to malignant and correlates directly with Gleason score and inversely with tumor differentiation, supporting its use as a diagnostic and prognostic marker [44]. On the other hand, some studies found no statistically significant relationship between p53 expression and increasing Gleason grade [10, 11]. These observations were also reinforced by the study by Wahid *et al.*, with high p53 expression scores being absent in low-grade tumors and a gradual increase in the intensity and percentage of positive nuclei with the transition to high-grade groups [38]. Also, a recent study reported similar data, identifying a statistically significant association between increased p53 immunoexpression and tumors classified as pT3–T4 [45]. In prostate cancer, a statistical association was identified between increased p53 expression and the presence of LVI [45], although there are limited data regarding p53 expression reported in invasive prostate tumors.

Ki67 is one of the most widely used markers of cell proliferative activity and provides relevant information on the biological aggressiveness of malignant tumors, including prostate carcinoma [20, 46]. It is a nuclear protein expressed in all phases of the active cell cycle (G1, S, G2 and M), but is absent in the G0 phase, therefore, immunohistochemistry for Ki67 represents an index of tumor proliferation, widely used in oncology to assess the degree of biological aggressiveness [47]. The *WHO* (2022) recommends optional reporting of Ki67 in prostate carcinoma, noting that increased expression may suggest neoplasia with aggressive behavior, but it does not yet constitute an official classification criterion [20].

In our study, Ki67 reactions were present in all cases with significant or borderline associations with PAA type, high grade, PNI and LVI. We also found rare labeled epithelial cells in non-cancerous areas, these differences being noted in other studies where reactions were superior in PAA compared to hyperplastic or normal prostate [48, 49].

Over time, extensive studies have been conducted on Ki67 expression in prostate cancer. Thus, Ki67 is significantly correlated with poor prognosis of localized prostate cancer, with decreased OS and high recurrence rate [18]. Ki67 seems to have additional prognostic value compared to Gleason score and PSA value, being an independent predictor of recurrence and mortality specific to prostate cancer [50]. A relationship between increased immunostaining of the marker and the risk of distant metastasis is suggested, Ki67 evaluation being useful for individualizing therapeutic strategies [51]. Significant correlation of Ki67 expression with Gleason score, tumor stage and therapeutic failure increases the practical value of the marker [52]. Recently, studies have extended the utility of Ki67 beyond prognosis. Albuquerque-Castro *et al.* demonstrated that an "immunoscoring" for Ki67 can improve risk stratification in prostate cancer [53].

In our study, there were significant positive linear correlations of the three markers analyzed, which seem consistent with data from the literature [6]. Cyclin D1 reactions are described as being consistent with the Ki67 proliferation index [27], and the tandem P53 and Ki67 is proposed as an adjuvant for the histological and prognostic classification of malignant prostatic lesions [36] and may provide additional information compared to conventional histology [54]. These results support the predictive value of IHC panels in the evaluation of patients with prostate carcinoma [29].

## Conclusions

Cyclin D1, P53 and Ki67 expression were associated with PAA aggressiveness parameters, although the reaction values were variable even in the relatively homogeneous group analyzed. The negative P53 reactions present in all analyzed categories seem to indicate a distinct category of PAA, the same aspect being valid for cases with consistent P53 marker. The positive correlation of Ki67 with Cyclin D1 and P53 indicates their involvement in tumor proliferation. Overall, the analyzed panel is useful for the individual rather than group assessment of PAA and can complement criteria considered for specific oncological therapy.

**Conflict of interests**

The authors declare that they have no conflict of interests.

## References


[1] Giona S. The epidemiology of prostate cancer. In: Bott SRJ, Ng KL (eds). Prostate cancer [Internet]. Exon Publications, Brisbane, Australia, 2021 May 27, Chapter 1. https://doi.org/10.36255/exonpublications.prostatecancer.epidemiology.2021 PMID: 34181376

[2] Siegel RL, Kratzer TB, Giaquinto AN, Sung H, Jemal A. Cancer statistics, 2025. CA Cancer J Clin, 2025, 75(1):10–45. https://doi.org/10.3322/caac.21871 PMID: 39817679 PMCID: PMC11745215

[3] Kehinde EO, Al Maghrebi M, Anim JT. The importance of determining the aggressiveness of prostate cancer using serum and tissue molecular markers. Can J Urol, 2008, 15(2):3967–3974. PMID: 18405443

[4] Glaviano A, Singh SK, Lee EHC, Okina E, Lam HY, Carbone D, Reddy EP, O'Connor MJ, Koff A, Singh G, Stebbing J, Sethi G, Crasta KC, Diana P, Keyomarsi K, Yaffe MB, Wander SA, Bardia A, Kumar AP. Cell cycle dysregulation in cancer. Pharmacol Rev, 2025, 77(2):100030. https://doi.org/10.1016/j.pharmr.2024.100030 PMID: 40148026

[5] Finn RS, Martin M, Rugo HS, Jones S, Im SA, Gelmon K, Harbeck N, Lipatov ON, Walshe JM, Moulder S, Gauthier E, Lu DR, Randolph S, Diéras V, Slamon DJ. Palbociclib and Letrozole in advanced breast cancer. N Engl J Med, 2016, 375(20):1925–1936. https://doi.org/10.1056/NEJMoa1607303 PMID: 27959613

[6] Drobnjak M, Osman I, Scher HI, Fazzari M, Cordon-Cardo C. Overexpression of cyclin D1 is associated with metastatic prostate cancer to bone. Clin Cancer Res, 2000, 6(5):1891–1895. PMID: 10815912

[7] Fleischmann A, Rocha C, Saxer-Sekulic N, Zlobec I, Sauter G, Thalmann GN. High-level cytoplasmic cyclin D1 expression in lymph node metastases from prostate cancer independently predicts early biochemical failure and death in surgically treated patients. Histopathology, 2011, 58(5):781–789. https://doi.org/10.1111/j.1365-2559.2011.03800.x PMID: 21438905

[8] Carneiro A, Barbosa ÁRG, Takemura LS, Kayano PP, Moran NKS, Chen CK, Wroclawski ML, Lemos GC, da Cunha IW, Obara MT, Tobias-Machado M, Sowalsky AG, Bianco B. The role of immunohistochemical analysis as a tool for the diagnosis, prognostic evaluation and treatment of prostate cancer: a systematic review of the literature. Front Oncol, 2018, 8:377. https://doi.org/10.3389/fonc.2018.00377 PMID: 30280090 PMCID: PMC6153326

[9] Ofner H, Kramer G, Shariat SF, Hassler MR. *TP53* deficiency in the natural history of prostate cancer. Cancers (Basel), 2025, 17(4):645. https://doi.org/10.3390/cancers17040645 PMID: 40002239 PMCID: PMC11853097

[10] Sulik M, Guzińska-Ustymowicz K, Darewicz B, Sulik A. Study of p53 protein expression in prostate cancer. Folia Histochem Cytobiol, 2001, 39(Suppl 2):197–198. PMID: 11820605

[11] Madani SH, Ameli S, Khazaei S, Kanani M, Izadi B. Frequency of Ki-67 (MIB-1) and P53 expressions among patients with prostate cancer. Indian J Pathol Microbiol, 2011, 54(4):688–691. https://doi.org/10.4103/0377-4929.91492 PMID: 22234091

[12] Corti M, Lorenzetti S, Ubaldi A, Zilli R, Marcoccia D. Endocrine disruptors and prostate cancer. Int J Mol Sci, 2022, 23(3):1216. https://doi.org/10.3390/ijms23031216 PMID: 35163140 PMCID: PMC8835300

[13] Şerban RE, Boldeanu MV, Florescu DN, Ionescu M, Şerbănescu MS, Boldeanu L, Florescu MM, Stepan MD, Obleagă VC, Constantin C, Popescu DM, Streba CT, Vere CC. Comparison between substance P and calcitonin gene-related peptide and their receptors in colorectal adenocarcinoma. J Clin Med, 2024, 13(18):5616. https://doi.org/10.3390/jcm13185616 PMID: 39337103 PMCID: PMC11432560

[14] Şerban RE, Stepan MD, Florescu DN, Boldeanu MV, Florescu MM, Şerbănescu MS, Ionescu M, Streba L, Drăgoescu NAM, Christopher P, Obleagă VC, Constantin C, Vere CC. Expression of calcitonin gene-related peptide and calcitonin receptor-like receptor in colorectal adenocarcinoma. Int J Mol Sci, 2024, 25(8):4461. https://doi.org/10.3390/ijms25084461 PMID: 38674047 PMCID: PMC11050384

[15] Knudsen KE, Cavenee WK, Arden KC. D-type cyclins complex with the androgen receptor and inhibit its transcriptional transactivation ability. Cancer Res, 1999, 59(10):2297–2301. PMID: 10344732

[16] Tsai H, Morais CL, Alshalalfa M, Tan HL, Haddad Z, Hicks J, Gupta N, Epstein JI, Netto GJ, Isaacs WB, Luo J, Mehra R, Vessella RL, Karnes RJ, Schaeffer EM, Davicioni E, De Marzo AM, Lotan TL. Cyclin D1 loss distinguishes prostatic small-cell carcinoma from most prostatic adenocarcinomas. Clin Cancer Res, 2015, 21(24):5619–5629. https://doi.org/10.1158/1078-0432.CCR-15-0744 PMID: 26246306 PMCID: PMC4681606

[17] Nakamura Y, Felizola SJA, Kurotaki Y, Fujishima F, McNamara KM, Suzuki T, Arai Y, Sasano H. Cyclin D1 (CCND1) expression is involved in estrogen receptor beta (ER$\beta$) in human prostate cancer. Prostate, 2013, 73(6):590–595. https://doi.org/10.1002/pros.22599 PMID: 23060014

[18] Berlin A, Castro-Mesta JF, Rodriguez-Romo L, Hernandez-Barajas D, González-Guerrero JF, Rodríguez-Fernández IA, González-Conchas G, Verdines-Perez A, Vera-Badillo FE. Prognostic role of Ki-67 score in localized prostate cancer: a systematic review and meta-analysis. Urol Oncol, 2017, 35(8):499–506. https://doi.org/10.1016/j.urolonc.2017.05.004 PMID: 28648414

[19] Song Z, Zhou Q, Zhang JL, Ouyang J, Zhang ZY. Marker Ki-67 is a potential biomarker for the diagnosis and prognosis of prostate cancer based on two cohorts. World J Clin Cases, 2024, 12(1):32–41. https://doi.org/10.12998/wjcc.v12.i1.32. Erratum in: World J Clin Cases, 2024, 12(36):6950–6951. https://doi.org/10.12998/wjcc.v12.i36.6950 PMID: 38292624 PMCID: PMC10824173

[20] World Health Organization (WHO) Classification of Tumours Editorial Board. Urinary and male genital tumors. WHO Classification of Tumours, 5th edition, International Agency for Research on Cancer (IARC) Press, Lyon, France, 2022. https://publications.iarc.who.int/Book-And-Report-Series/Who-Classification-Of-Tumours/Urinary-And-Male-Genital-Tumours-2022

[21] Testa U, Castelli G, Pelosi E. Cellular and molecular mechanisms underlying prostate cancer development: therapeutic implications. Medicines (Basel), 2019, 6(3):82. https://doi.org/10.3390/medicines6030082 PMID: 31366128 PMCID: PMC6789661

[22] Podeanu MA, Vintilescu ŞB, Sandu RE, Ionele CM, Niculescu CE, Florescu MM, Şelaru EL, Stepan MD. Ferritin as an inflammatory marker in pediatric metabolic syndrome: links to obesity and liver ultrasound alterations. Int J Mol Sci, 2025, 26(8):3793. https://doi.org/10.3390/ijms26083793 PMID: 40332421 PMCID: PMC12027671

[23] Ciurea M, Ciurea R, Popa D, Pârvănescu H, Marinescu D, Vrabete M. Sinusoidal hemangioma of the arm: case report and review of literature. Rom J Morphol Embryol, 2011, 52(3):915–918. PMID: 21892538

[24] Grecu AF, Popa DG, Lungulescu CV, Ciucă EM, Camen A, Marinescu D, Nica O, Busuioc CJ, Ifrim Chen F, Ciurea ME. Histological findings from rat calvaria defect augmented with platelet-rich fibrin by using two consecutive periosteal incisions. Rom J Morphol Embryol, 2019, 60(1):111–118. PMID: 31263834

[25] Sherr CJ. D-type cyclins. Trends Biochem Sci, 1995, 20(5):187–190. https://doi.org/10.1016/s0968-0004(00)89005-2 PMID: 7610482

[26] Petre-Draviam CE, Cook SL, Burd CJ, Marshall TW, Wetherill YB, Knudsen KE. Specificity of cyclin D1 for androgen receptor regulation. Cancer Res, 2003, 63(16):4903–4913. PMID: 12941814

[27] Comstock CES, Revelo MP, Buncher CR, Knudsen KE. Impact of differential cyclin D1 expression and localisation in prostate cancer. Br J Cancer, 2007, 96(6):970–979. https://doi.org/10.1038/sj.bjc.6603615 PMID: 17375037 PMCID: PMC2360090

[28] Mohammed AA, Ibrahim HM, Atwa HA, Elshentenawy A, Elwan A. Impact of cyclin D1 and DJ-1 on diagnosis, clinico-pathological features and outcome in prostate cancer and benign prostatic hyperplasia. Forum Clin Oncol, 2019, 10(2):15–25. https://doi.org/10.2478/fco-2019-0005

[29] Zedan EM, Morgan NM, El-Sawi RM, Youssef SA. The study of Mucin stains and Cyclin D1 expression in benign prostatic hyperplasia and prostatic adenocarcinoma (Immunohistochemical study). Benha Med J. 2024, 41(6):73–86. https://doi.org/10.21608/bmfj.2024.267338.2011

[30] Yu CC, Lin VC, Huang CY, Liu CC, Wang JS, Wu TT, Pu YS, Huang CH, Huang CN, Huang SP, Bao BY. Prognostic significance of cyclin D1 polymorphisms on prostate-specific antigen recurrence after radical prostatectomy. Ann Surg Oncol, 2013, 20(Suppl 3):S492–S499. https://doi.org/10.1245/s10434-013-2869-x PMID: 23354566

[31] Aaltomaa S, Kärjä V, Lipponen P, Isotalo T, Kankkunen JP, Talja M, Mokka R. Expression of Ki-67, cyclin D1 and apoptosis

markers correlated with survival in prostate cancer patients treated by radical prostatectomy. Anticancer Res, 2006, 26(6C): 4873–4878. PMID: 17214354

[32] Ding GX, Liu J, Feng CC, Jiang HW, Xu JF, Ding Q. Slug regulates Cyclin D1 expression by ubiquitin-proteasome pathway in prostate cancer cells. Panminerva Med, 2012, 54(3):219–223. PMID: 22801439

[33] Zhou J, Lai Y, Peng S, Tang C, Chen Y, Li L, Huang H, Guo Z. Comprehensive analysis of *TP53* and *SPOP* mutations and their impact on survival in metastatic prostate cancer. Front Oncol, 2022, 12:957404. https://doi.org/10.3389/fonc.2022.957404 PMID: 36119488 PMCID: PMC9471084

[34] Maddah MM, Hedayatizadeh-Omran A, Moosazadeh M, Alizadeh-Navaei R. Evaluation of the prognostic role of *TP53* gene mutations in prostate cancer outcome: a systematic review and meta-analysis. Clin Genitourin Cancer, 2024, 22(6):102226. https://doi.org/10.1016/j.clgc.2024.102226 PMID: 39393313

[35] Zhang W, Dong Y, Sartor O, Zhang K. Deciphering the increased prevalence of *TP53* mutations in metastatic prostate cancer. Cancer Inform, 2022, 21:11769351221087046. https://doi.org/10.1177/11769351221087046 PMID: 35392296 PMCID: PMC8980432

[36] Verma R, Gupta V, Singh J, Verma M, Gupta G, Gupta S, Sen R, Ralli M. Significance of p53 and Ki-67 expression in prostate cancer. Urol Ann, 2015, 7(4):488–493. https://doi.org/10.4103/0974-7796.158507 PMID: 26692671 PMCID: PMC4660702

[37] Kudahetti S, Fisher G, Ambroisine L, Foster C, Reuter V, Eastham J, Møller H, Kattan MW, Cooper CS, Scardino P, Cuzick J, Berney DM. p53 immunochemistry is an independent prognostic marker for outcome in conservatively treated prostate cancer. BJU Int, 2009, 104(1):20–24. https://doi.org/10.1111/j.1464-410X.2009.08407.x PMID: 19239456

[38] Wahid Z, Jaffer R, Khalid S, Khan HA, Riaz S. Expression of p53 protein in prostate carcinoma and its correlation with Gleason grade. J Coll Physicians Surg Pak, 2020, 30(6):643–645. https://doi.org/10.29271/jcpsp.2020.06.643 PMID: 32703352

[39] Ando K, Oki E, Saeki H, Yan Z, Tsuda Y, Hidaka G, Kasagi Y, Otsu H, Kawano H, Kitao H, Morita M, Maehara Y. Discrimination of p53 immunohistochemistry-positive tumors by its staining pattern in gastric cancer. Cancer Med, 2015, 4(1):75–83. https://doi.org/10.1002/cam4.346 PMID: 25354498 PMCID: PMC4312120

[40] Schlomm T, Iwers L, Kirstein P, Jessen B, Köllermann J, Minner S, Passow-Drolet A, Mirlacher M, Milde-Langosch K, Graefen M, Haese A, Steuber T, Simon R, Huland H, Sauter G, Erbersdobler A. Clinical significance of p53 alterations in surgically treated prostate cancers. Mod Pathol, 2008, 21(11): 1371–1378. https://doi.org/10.1038/modpathol.2008.104 PMID: 18552821

[41] Stopsack KH, Salles DC, Vaselkiv JB, Grob ST, Mucci LA, Lotan TL. p53 immunohistochemistry to identify very high-risk primary prostate cancer: a prospective cohort study with three decades of follow-up. Eur Urol Oncol, 2023, 6(1):110–112. https://doi.org/10.1016/j.euo.2021.12.003 PMID: 34969655 PMCID: PMC9555222

[42] Kallakury BV, Figge J, Ross JS, Fisher HA, Figge HL, Jennings TA. Association of p53 immunoreactivity with high Gleason tumor grade in prostatic adenocarcinoma. Hum Pathol, 1994, 25(1): 92–97. https://doi.org/10.1016/0046-8177(94)90177-5 PMID: 7508886

[43] Petrescu A, Mârzan L, Codreanu O, Niculescu L. Immunohistochemical detection of p53 protein as a prognostic indicator in prostate carcinoma. Rom J Morphol Embryol, 2006, 47(2):143–146. PMID: 17106522

[44] Bhat G, Ahamed MA, Saroha N, Prasad G. Clinicopathological correlation of p53 expression in benign prostatic hyperplasia and prostate adenocarcinoma. Indian J Pathol Oncol, 2022, 9(1):60–64. https://doi.org/10.18231/j.ijpo.2022.015

[45] Gesztes W, Schafer C, Young D, Fox J, Jiang J, Chen Y, Kuo HC, Mwamukonda KB, Dobi A, Burke AP, Moul JW, McLeod DG, Rosner IL, Petrovics G, Tan SH, Cullen J, Srivastava S, Sesterhenn IA. Focal p53 protein expression and lymphovascular invasion in primary prostate tumors predict metastatic progression. Sci Rep, 2022, 12(1):5404. https://doi.org/10.1038/s41598-022-08826-5. PMID: 35354846 PMCID: PMC8967869

[46] Niculescu ŞA, Grecu DC, Simionescu CE, Niculescu EC, Stepan MD, Stepan AE. Immunoexpression of Ki67, p53 and cyclin D1 in osteosarcomas. Rom J Morphol Embryol, 2021, 62(3):743–750. https://doi.org/10.47162/RJME.62.3.11 PMID: 35263402 PMCID: PMC9019677

[47] Tretiakova MS, Wei W, Boyer HD, Newcomb LF, Hawley S, Auman H, Vakar-Lopez F, McKenney JK, Fazli L, Simko J, Troyer DA, Hurtado-Coll A, Thompson IM Jr, Carroll PR, Ellis WJ, Gleave ME, Nelson PS, Lin DW, True LD, Feng Z, Brooks JD. Prognostic value of Ki67 in localized prostate carcinoma: a multi-institutional study of >1000 prostatectomies. Prostate Cancer Prostatic Dis, 2016, 19(3):264–270. https://doi.org/10.1038/pcan.2016.12 PMID: 27136741 PMCID: PMC5536893

[48] di Sant'Agnese PA. Neuroendocrine differentiation in carcinoma of the prostate. Diagnostic, prognostic, and therapeutic implications. Cancer, 1992, 70(1 Suppl):254–268. https://doi.org/10.1002/1097-0142(19920701)70:1+<254::aid-cncr2820701312>3.0.co;2-e PMID: 1350941

[49] Bakna M, Malik R, Jain P, Jain R, Jain A. Diagnostic and prognostic role of Ki-67 and cytokeratin-5 expression in BPH and carcinoma prostate. Int J Med Res Rev, 2016, 4(8):1484–1492. https://doi.org/10.17511/ijmrr.2016.i08.32

[50] Berney DM, Gopalan A, Kudahetti S, Fisher G, Ambroisine L, Foster CS, Reuter V, Eastham J, Moller H, Kattan MW, Gerald W, Cooper C, Scardino P, Cuzick J. Ki-67 and outcome in clinically localised prostate cancer: analysis of conservatively treated prostate cancer patients from the Trans-Atlantic Prostate Group study. Br J Cancer, 2009, 100(6):888–893. https://doi.org/10.1038/sj.bjc.6604951 PMID: 19293807 PMCID: PMC2661778

[51] Zellweger T, Günther S, Zlobec I, Savic S, Sauter G, Moch H, Mattarelli G, Eichenberger T, Curschellas E, Rüfenacht H, Bachmann A, Gasser TC, Mihatsch MJ, Bubendorf L. Tumour growth fraction measured by immunohistochemical staining of Ki67 is an independent prognostic factor in preoperative prostate biopsies with small-volume or low-grade prostate cancer. Int J Cancer, 2009, 124(9):2116–2123. https://doi.org/10.1002/ijc.24174 PMID: 19117060

[52] Mohamed AA, Abbas MY, Alharbi H, Babiker AY. Assessment of expression of Ki-67 in benign and malignant prostatic lesions among Sudanese patients. Open Access Maced J Med Sci, 2018, 6(10):1809–1812. https://doi.org/10.3889/oamjms.2018.309 PMID: 30455753 PMCID: PMC6236043

[53] Albuquerque-Castro Â, Macedo-Silva C, Oliveira-Sousa R, Constâncio V, Lobo J, Carneiro I, Henrique R, Jerónimo C. Redefining prostate cancer risk stratification: a pioneering strategy to estimate outcome based on Ki67 immunoscoring. Biomark Res, 2024, 12(1):75. https://doi.org/10.1186/s40364-024-00627-4. Erratum in: Biomark Res, 2024, 12(1):121. https://doi.org/10.1186/s40364-024-00670-1 PMID: 39090707 PMCID: PMC11295892

[54] Ruhela S, Chadha S, Agarwal S. Analyzing the Gleason's score, Ki-67 index and p53 expression in prostate. Int J Acad Med Pharm, 2025, 7(3):110–113. https://doi.org/10.47009/jamp.2025.7.3.22

***Corresponding authors***

Bianca Cătălina Andreiana, Teaching Assistant, MD, PhD, Department of Pathology, University of Medicine and Pharmacy of Craiova, 66 1 May Avenue, 200628 Craiova, Dolj County, Romania; Phone/Fax +40251–599 228, e-mail: bianca.andreiana@umfcv.ro

Oana Iulia Creţu, Teaching Assistant, MD, PhD, Department of Pathology, University of Medicine and Pharmacy of Craiova, 66 1 May Avenue, 200628 Craiova, Dolj County, Romania; Phone/Fax +40251–599 228, e-mail: oana.cretu@umfcv.ro

*Received: December 12, 2025* *Accepted: December 28, 2025*