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Protein C Inhibitor as a Diagnostic Marker: Immunohistochemical Expression in Prostatic Adenocarcinoma and Benign Prostatic Hyperplasia

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Abstract

***Background**:* This study explores the immunohistochemical expression of protein C inhibitor (PCI) in patients with prostatic adenocarcinoma (PCa) and benign prostatic hyperplasia (BPH).

***Methods and Results**:* Our study involved 56 prostate samples morphologically diagnosed as PCa and 30 BPH samples. An immunohistochemical analysis of the PCI expression was performed. Our analysis encompassed variables including age, serum prostate-specific antigen (PSA), local and distant metastasis, PCI staining, and morphological features such as Gleason Score and Grade Group. In addition to descriptive statistics, Spearman's rank correlation test was performed. All patients with BPH had no staining or low staining for PCI. 60% of PCa patients had moderate or strong staining intensity for PCI. There was no correlation between PCI expression and clinicopathological parameters, including PCI expression and PSA, Gleason Score, and Grade Group. A negative correlation was observed between age at diagnosis and PCI expression.

***Conclusion**:* Our study did not find a statistically significant correlation between PCI and PCa. However, we report that in 60% of our PCa cases, there is a moderate and high-intensity expression for PCI.**(International Journal of Biomedicine. 2024;14(3):441-447.)**

Keywords: prostate • cancer • biomarkers • prostate-specific antigen • protein C inhibitor

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Abbreviations

BPH, benign prostatic hyperplasia; **DRE**, digital rectal examination; **PCa**, prostatic adenocarcinoma; **PCI**, protein C inhibitor; **PSA**, prostate-specific antigen.

Introduction

Prostatic adenocarcinoma (PCa) is a leading cause of cancer-related morbidity and mortality among men. Early detection and diagnosis are critical for effective management and improving patient outcomes. Current screening methods have limitations, including false positives and overdiagnosis. The most widely adopted method for screening PCa is the measurement of serum prostate-specific antigen (PSA) in conjunction with the digital rectal examination (DRE).[1] Several studies have reported a decrease in mortality when there is a screening system, including the 16-year randomized study by Hugosson et al. with European screening data.[2-6] In this case, the authors observed a rate ratio of 0.80 (95% CI 0.72-0.89) for mortality.[2] However, such observations are not uniform, and in some study reports, no change in mortality has been observed.[7]

Moreover, systematic screening using PSA testing and DRE with biopsy confirmation is often associated with problems of overdiagnosis, leading to over-treatment of low-risk patients.[7-9] Additionally, high PSA levels can also indicate other non-cancerous conditions, such as benign prostatic hyperplasia (BPH) and inflammation, or may be caused by dietary changes.[9] As such, new, more specific biomarkers are needed to stratify risk better. [10-12]

One potential candidate for an operationally simple biomarker is a protein C inhibitor (PCI). PCI belongs to the SERPIN family and is involved in the inhibition of a wide variety of proteins, affecting not only protein C but also the processes of fibrinolysis, fertilization, healing, and metastasis, as a regulator of coagulation, and other proteolytic processes. [13] Differences in PCI expression have been reported between healthy, benign, and malignant prostatic tissues.[14-16] Cao et al.,[16] in their analyses of benign and malignant prostatic tissues, detected PCI in all tissues except in high-grade prostatic tumors, suggesting that decreased PCI expression, in conjunction with continued protease production, may promote invasive growth. This has also been observed in ovarian cancer tissues, where significantly lower levels of PCI were found in carcinomas compared to borderline tumors and in omental metastases compared to carcinomas.[17]

Studies have reported using mass spectrometry (MS)-based proteomics methods to quantify PCI or its fragments, such as SELDI mass spectrometry, which was patented for this purpose in 2013.[18] MS techniques boast a high level of precision and are essential in research settings. However, MS is a complex, time-consuming, and expensive method to apply in everyday clinical practice.[19] Further studies are needed to translate the results of proteomics-based studies to more low-cost and accessible methods.

This study aimed to examine PCI expression using immunohistochemistry in patients with BPH and PCa. Within the carcinoma group, our study compares PCI expression between patients regarding PSA, Gleason Score, Grade group, and metastasis, thus investigating the relationship between PCI and case severity.

Materials and Methods

The data for this study was collected at the Urology Clinic of the University Clinical Centre of Kosovo. To protect patient privacy, we replaced patient identifiers with study numbers. The study involved 56 prostate samples morphologically diagnosed as PCa and 30 BPH prostate samples. None of the patients had undergone preoperative hormonal therapy or radiotherapy. Approval for the study was obtained from the Ethics Committee of the Faculty of Medicine, University of Prishtina.

Benign tissue samples were obtained through transurethral prostate resection, while prostate cancer tissue samples were obtained from transrectal ultrasound-guided prostate biopsy or radical prostatectomy. In the case of radical prostatectomy, prostate cancer patients had been previously diagnosed using transrectal prostate biopsy. Following the surgical removal, sections measuring 5 μm in thickness were sliced from paraffin blocks that held prostatic tissue fixed in 10% buffered formalin. These sections were then deparaffinized and subjected to hematoxylin and eosin staining for analysis under a light microscope.

Immunohistochemistry

Immunohistochemical analysis of the expression of PCI (ab13125 polyclonal, 1:100 dilution, Abcam Cambridge) in PCa and BPH was performed using the EnVision Flex-system on a DakoTechMate™ immunohistochemical autostainer. As a negative control, the primary antibody was omitted. PCI expression was noted in the cytoplasm of epithelial cells lining the prostatic acini. Immunohistochemical staining was assessed by analyzing specific areas of prostate tissue referred to as “hot spots.”

The immunohistochemical staining was analyzed semi-quantitatively according to the grading standard of 0-3, where 0 denotes no staining, 1- up to 25% of prostate tissue is positive, 2 indicates between 25 and 50%, and 3 signifies more than 50% positivity in prostate tissue. The staining intensity was evaluated as follows: 0 (negative), 1 (weak positivity, observed at a magnification of 200x), 2 (moderate, observed at a magnification of 100x), and 3 (strong, observed at a magnification of 20x).

Statistical Analysis

We conducted a descriptive analysis of clinicopathological features across two study cohorts: patients diagnosed with BPH and those diagnosed with PCa. Our analysis encompassed a range of crucial variables, including age at diagnosis, PSA levels, percentage of positive staining for PCI, staining intensity of PCI, presence of bone metastasis, presence of local metastasis, Gleason Score, and Grade group. For continuous variables, such as age at diagnosis and PSA levels, we computed mean (M) and standard deviation (SD) or median (Me) and interquartile range (IQR) depending on the normality of the distribution. For ordinal and binary variables, proportions were calculated. Furthermore, to elucidate potential relationships between clinicopathological features and staining characteristics, specifically staining intensity and percentage, Spearman’s rank correlation test was conducted for both BPH and PCa patient cohorts. This allowed for a nuanced exploration of the clinicopathological landscape within each study group and provided insights into potential associations between clinicopathological variables and staining attributes, thus enriching our understanding of the underlying pathological mechanisms in these conditions. All analysis was performed with Stata BE Statistical Software: Release 18. College Station, TX: StataCorp LLC.

Results

Samples were taken from 30 patients with BPH and 56 with PCa. We carried out immunohistochemical staining of sampled tissues in both groups (Figure 1). PCI expression was graded according to the scale defined in the methods section. The staining percentage is the percentage of tissue that is stained, and staining intensity denotes the level of magnification at which staining is observed. Examples of strong and moderate to weak staining for BPH (Figure 1a-b) and PCa (Figure 1c-d) are presented.

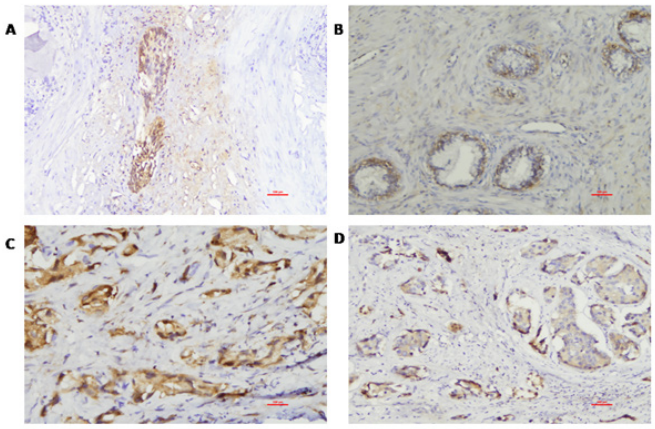

Fig. 1. *PCI expression in prostatic tissue. A. PCI strong cytoplasmic staining in BPH (100x magnification). B. PCI moderate and weak cytoplasmic staining in BPH (100x magnification). C. PCI strong cytoplasmic staining in PCa with Gleason Score 6 and Gleason Grade 3+3 (Grade Group 1) (200x magnification). D. PCI weak and negative cytoplasmic staining in PCa with Gleason Score 7 and Gleason Grade 3+4 (Grade Group 2) (100x magnification).*

In the group of patients with BPH (Table 1), the average age was 68.9±7.6 years. The median PSA value in this group was 3.8 (IQR: 2.5-5.1), below the established reference value of 4 ng/mL.[20] This is consistent with the diagnosis of BPH, where a lower PSA level is expected,[21,22] although it is worth noting that some recorded values were above the reference range. In terms of the presence of PCI, 40% of the samples had a staining percentage of 0, denoting no detection of PCI, while the remaining 60% had a staining percentage of 1, denoting less than or equal to 25% of cells demonstrating the presence of PCI. Only 2 samples (6.7%) had strong PCI staining intensity, with 40% showing no PCI staining.

Table 1.

Clinicopathological features of patients with BPH.

Features	n (%)
Age at diagnosis, years (M±SD)	68.9 (7.6)
PSA (ng/mL) Me (IQR)	3.8 (2.4-5.1)
[a]PCI Staining % 0 1 2 3	 12 (40.0) 18 (60.0) 0 (0.0) 0 (0.0)
[b]PCI Staining intensity 0 1 2 3	 12 (40.0) 4 (13.3) 12 (40.0) 2 (6.7)
[a] 0 = negative, 1 = ≤25%, 2 = >25 - ≤ 50%, 3 = >50%, [b] 0 = negative, 1 = weak, 2 = moderate, 3 = strong	

Of the 56 samples that had been morphologically diagnosed as PCa (Table 2), the average age of 73.8 was higher than in the BPH group, and the median PSA value was 60.8, considerably above the reference value, with a broad interquartile range (32.0-95.8). Most samples showed the presence of PCI, with only 20 samples being negative for PCI (35.7%). Of the positive samples, 22 (39.3%) showed less than or equal to 25% staining, 13 (23.2%) showed between 25 and 50% staining and 1 sample had more than 50% staining (1.8%). Only 1 sample had low staining intensity (1.8%), while 18 (32.1%) and 17 (30.4%) had moderate and strong staining, respectively. The majority of cases were positive for local metastasis (46, 82.1%), and bone metastasis was also observed in 28 more advanced cases (50%). The largest group of prostate cancer patients was those with a Gleason Score of 7, which constituted 18 patients, or 32.1%. Grade group 5 tumors were most common in this patient sample (19, 33.9%).

Table 2.

Clinicopathological features of patients with PCa.

Features	n (%)
Age at diagnosis, years (M±SD)	73.8 (8.5)
PSA (ng/mL) Me (IQR)	60.8 (32.0-95.8)
[a]PCI Staining % 0 1 2 3	 20 (35.7) 22 (39.3) 13 (23.2) 1 (1.8)
[b]PCI Staining intensity 0 1 2 3	 20 (35.7) 1 (1.8) 18 (32.1) 17 (30.4)
Presence of local metastasis	46 (82.1)
Presence of bone metastasis	28 (50.0)
Gleason Score 6 7 8 9 10	 8 (14.3) 18 (32.1) 11 (19.6) 9 (16.1) 10 (17.9)
Grade Group 1 2 3 4 5	 8 (14.3) 10 (17.9) 8 (14.3) 11 (19.6) 19 (33.9)
[a] 0 = negative, 1 = ≤25%, 2 = >25 - ≤ 50%, 3 = >50%, [b] 0 = negative, 1 = weak, 2 = moderate, 3 = strong	

We carried out Spearman correlation analysis in both patient categories to determine whether PCI expression (via immunohistochemical staining percentage and staining intensity) was associated with other clinical and morphological features.

In BPH patients, we examined the correlation between increased age and PCI staining percentage and intensity (Table 3). In both cases, no correlation was observed (Spearman coefficient >0.01), with p-values of 0.631 and 0.823, respectively, suggesting no overall correlation

between advanced age and PCI expression as detected by immunohistochemical methods. In the case of PSA, similarly, no significant correlation was observed between PCI staining percentage or staining intensity and PSA concentration in serum.

Table 3.

Correlations between PCI staining and clinicopathological features in patients with BPH.

Features	PCI staining %		PCI staining intensity	
	Spearman coefficient	*P*-value	Spearman coefficient	*P*-value
Age at diagnosis	0.091	0.631	0.042	0.823
PSA	-0.024	0.901	-0.096	0.611
PSA=Prostate Specific Antigen, PCI=Protein C Inhibitor				

One key aim of this study was to examine the potential of PCI to act as a marker of case severity, building on other studies that report lower PCI expression in more advanced tumors.[23] As such, we examined the Spearman correlation values for a series of clinicopathological characteristics: age, PSA level, metastasis, Gleason Score, and Grade group (Table 4). We found that only age at diagnosis and PCI staining had a moderate, statistically significant inverse correlation, implying that strong PCI staining intensity is less likely in older patients. The Gleason Score and Grade groups did not show any statistically significant relationship between PCI staining percentage and intensity in PCa.

Table 4.

Correlations between PCI staining and clinicopathological features in patients with PCa.

Features	PCI staining %		PCI staining intensity	
	Spearman coefficient	*P*-value	Spearman coefficient	*P*-value
Age at diagnosis	-0.227	0.092	-0.425	0.001
PSA	-0.002	0.987	-0.019	0.887
Gleason Score	-0.081	0.551	0.005	0.970
Grade Group	-0.078	0.564	0.013	0.922
PSA= Serum Prostate Specific Antigen, PCI=Protein C Inhibitor				

Discussion

Summary of Results

Our study investigated the relationship between PCI expression and clinical and morphological characteristics in patients with BPH and PCa. Overall, we did not observe a significant correlation, positive or negative, between PCI expression—either by PCI staining percentage or intensity—and BPH. Moreover, there was no correlation between PCI and the Gleason Score or Grade Group in PCa. We observed that increased age at PCa diagnosis had a moderate inverse correlation with PCI expression in prostatic tissue.

Context

PCI is mainly produced in the liver,[24] although it is found in various tissues and low concentrations in plasma.[25-27] It fulfills several roles in the body, most notably as an inhibitor of activated protein C and other plasma proteases, which have been shown to increase tumor proliferation.[13,26,28-30] PCI is also linked to the inhibition of coagulation factors like thrombin and factor Xa,[31] suggesting its involvement in regulating coagulation.[32]

In studies of patients with prostate, kidney, and ovarian cancers, reduced PCI expression was observed in patients with more aggressive disease.[16,17,33] PCI was observed in basal and luminal cells of the prostatic epithelium. The reports included samples from patients with prostatic intraepithelial neoplasia, as well as a wide variety of tumor cells, including metastases, LNCaP, PC-3, and DU-145 cells; PCI was not observed in high-grade tumor cells. This previous research underlines an inverse association between case severity and PCI expression,[16] as has been reported for the likelihood of recurrence,[15,24] and PCI expression. However, several studies have observed PCI in all tissues studied, including high-grade PCa, suggesting it may not function as a tumor suppressor and introducing a potential role as a regulator of PSA or other proteases associated with PSA activation.[14]

Moreover, despite the results observed, which demonstrate a relationship between increased PCI expression and decreased metastasis or recurrence.[15-17,24,33] Fan et al.,[34] using qPCR to examine PCI expression and examine its potential to act as a predictor for survival in patients with gastric cancer, have reported a higher expression of PCI in gastric cancer patients and attributed this to a potential role in inhibiting CBL in the regulation of the PI3K/AKT/mTOR signaling pathway. In our study, we did not observe any significant correlation between PCI and prostatic adenocarcinoma; however, there was a moderate and high immunohistochemical expression of PCI in 60% of our sample, which we consider that in a larger sample could potentially result in statistical significance.

Mechanism

The inverse relationship between case severity and PCI expression, identified in other studies, may stem from various potential mechanisms. An inverse relationship with cancer progression has been observed with other serpin inhibitors besides PCI, marking them as potential targets for treatment.[28]

In addition to activated protein C, PCI forms complexes with other proteins, including urokinase, kallikrein, and PSA, which have been reported as markers for PCa.[14,35-,37] It has been reported that up to 10% of seminal PSA is in the form of an adduct complex with PCI.[38] PSA has been suggested to promote proliferation and the development of refractory tumors through the promotion of ARA70-AR-mediated cell growth, in addition to its protease activity.[39] Complex formation may, therefore, be associated with the regulation and inhibition of these proteins, effectively sequestering proteins that may be active in proliferation.

It has also been noted that PCI inhibits angiogenesis. Suzuki and Hayashi have linked PCI's antiangiogenetic role with the inhibition of heparin. In support of this idea, they

reported that the inhibitory effect of PCI was not observed in the presence of high concentrations of heparin.[29]

Strengths and Limitations

The potential of PCI as a biomarker for PCa diagnosis is still under question, and this study examines this potential in separate patient cohorts through immunohistochemistry. However, one drawback of the immunohistochemical approach is its inability to detect fragments of PCI. Some studies, such as that of Rosenzweig et al. have reported the activity of PCI fragments in modulating cancer activity, in this case, the N-terminal fragment of PCI.[15] This may be one reason we did not observe significant trends between PCI and prostate cancer. Future studies should account for this possibility in analysis.

The inability to detect a significant relationship in this study may also be related to sample size. A larger sample size would have enabled more inferential comparisons and potentially established a conclusive difference in PCI expression. Future studies should explore a different sampling approach, generating a fully matched case-control that would allow comparison between PCa, BPH, and healthy patients with similar clinical parameters. PCI has been reported to have the potential to function as a prognostic marker for biochemical recurrence following surgical interventions, including radical prostatectomy.[15] Our study would, therefore, be strengthened with longitudinal data to examine this possibility.

Implications

The primary method of PCa screening and diagnosis is the measurement of PSA levels and digital rectal examinations, with biopsy for confirmation. PSA screening programs have led, in many cases, to unnecessary treatment, including radical prostatectomies, which are associated with side effects and decreased quality of life. Recent guidelines, such as the EAU-EANM-ESTRO-ESUR-SIOG, have attempted to balance the costs and benefits of these programs, recommending screening in high-risk groups and excluding from screening men with less than 15 years of remaining life expectancy who are less likely to benefit from PCa treatment.[40] Moreover, PSA reliability has limitations. It has been reported that 8% of cases can only be identified by digital rectal examination, regardless of PSA values.[28] As such, there is a pressing need for next-generation biomarkers.

Novel biomarkers can aid in developing a more reliable and predictive method of diagnosis or prognosis, whether as a sole measured marker or in conjunction with an ensemble of other markers. PCI has also been featured in panels of biomarkers that can cooperatively predict PCa and its progression. Muazzam et al.[41] tested 12 protein biomarkers in serum samples of prostatic cancer patients and healthy controls and identified PCI as part of a panel of 5 biomarkers, along with GP5, ECM1, IGHG1, and THBS1. Overall, these markers gave a ROC AUC value of 0.91, suggesting potential as a diagnostic marker. However, the overall diagnostic accuracy sat at 0.77, and sensitivity and specificity were lower than in the initial discovery set of 12 markers.[41] A study by Wan et al.,[42] which used genomics methods, identified the gene coding PCI as a predictive variable for tumor immune dysfunction and exclusion, specifically as part of a composite variable that gave an AUC of 0.897 after 3 years. The potential future role of genomics and genetics techniques in biomarker identification and validation is beyond the scope of this article. Still, one example of this approach was reported by Hagelgans et al.,[43] who used nucleotide sequencing and PCR to examine methylation patterns of the SERPINA5 gene in normal and malignant prostate cells. They found differences in methylation between cell lines of normal and malignant cells, suggesting a prognostic role, but such an approach would need to be tested in real patient samples.

Our study analyses PCI expression by immunohistochemical staining and compares it with clinicopathological parameters in patients with BPH and prostatic adenocarcinoma. Such factors include age, PSA, Gleason Score, Grade Group, and local and distant metastasis. Our study did not observe a statistically significant correlation between Gleason Score, Grade Group, and PCI expression in PCa. Lower PCI expression was observed in prostatic adenocarcinoma patients with more advanced age at diagnosis (-0.425, P=0.001). We recommend further research with a larger pool of cases and matched controls to further examine this method of PCI analysis for case stratification.

Ethical Considerations

This study was approved by the Ethics Committee of the Faculty of Medicine, University of Prishtina "Hasan Prishtina."

Competing Interests

The authors declare that they have no competing interests.

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