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Immunological Consequences of rs5742621 Variant and IGF-1 in Modulating MIF, IL-27, and CXCL12 Expression in Individuals with Prostate Cancer

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Abstract

Prostate cancer (PCa) is the second most common cancer among men worldwide. Immunological biomarkers play a crucial role in disease diagnosis, progression, and treatment. This study aimed to investigate the relationship between the rs5742621 (A>G) genetic variant and serum levels of insulin-like growth factor 1 (IGF-1) and other cytokines in patients with PCa compared to healthy controls. A total of 204 patients with PCa (aged 48–80) and 196 healthy controls were enrolled. Blood samples were collected to genotype the rs5742621 variant using the tetra-primer amplification refractory mutation system-polymerase chain reaction (ARMS-PCR). Serum levels of IGF1, macrophage migration inhibitory factor (MIF), CXC motif chemokine ligand 12 (CXCL12), and interleukin-27 (IL-27) were measured using a sandwich immunoassay. The heterozygous GA genotype of rs5742621 was more prevalent among patients with PCa, while the AA genotype was more prevalent in healthy controls. Additionally, serum IGF-1 levels were significantly elevated in PCa patients (median: 110 pg/mL) compared to healthy controls (median: 65 pg/mL; $p < 0.001$). Similarly, MIF levels were higher in PCa patients (85 pg/mL vs. 45 pg/mL; $p < 0.001$), as were CXCL12 concentrations (140 pg/mL vs. 40 pg/mL; $p < 0.001$). In contrast, IL-27 levels were lower in PCa patients (40 pg/mL) than in controls (65 pg/mL; $p < 0.001$). ROC curve analysis demonstrated that CXCL12 and MIF both achieved perfect diagnostic accuracy (AUC = 1.0), while IGF1 showed excellent diagnostic performance (AUC = 0.979) and IL-27 showed good performance (AUC = 0.903). The GA genotype of rs5742621 was significantly associated with elevated IGF-1 levels and increased PCa risk, promoting cell proliferation, inhibiting apoptosis, and accelerating tumor growth. IGF-1 and related immunological markers may serve as promising diagnostic biomarkers and potential therapeutic targets for prostate cancer.

Keywords: Prostate cancer, rs5742621, ARM-PCR, IGF-1, MIF, CXCL12, IL27

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الدور المناعي للمتغير الجيني rs5742621 وعامل النمو الشبيه بالأنسولين IGF−1 في تغيير تعبير MIF و IL−27 و CXCL12 في الاشخاص المصابين بسرطان البروستات

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الخلاصة

يعد سرطان البروستات ثاني أكثر أنواع السرطان شيوعًا بين الرجال على مستوى العالم. تلعب المؤشرات المناعية دورًا حاسمًا في تشخيص المرض وتطوره وعلاجه. هدفت هذه الدراسة تحديد العلاقة بين المتغير الجيني (A>G) rs5742621 ومستويات عامل النمو الشبيه بالأنسولين−1 (IGF1) في مصل الدم، إلى جانب عدد من السيتوكينات لدى مرضى سرطان البروستات مقارنة بالأشخاص الأصحاء. شملت الدراسة 204 مريضًا بسرطان البروستات تتراوح أعمارهم بين 48–80 سنة و196 شخصًا من الأصحاء كمجموعة ضابطة. تم جمع عينات الدم لتحليل النمط الجيني للمتغير rs5742621 باستخدام تقنية تفاعل البوليمير التسلسلي بنظام التمهيد الرباعي المقاوم للطفرات كما تم قياس مستويات IGF1 ، والسيتوكينات الأخرى في مصل الدم باستخدام المقايسة المناعية من نوع الساندويتش .أظهرت النتائج أن النمط الجيني المتغاير GA للمتغير rs5742621 كان أكثر شيوعًا بين مرضى سرطان البروستات، في حين ساد النمط الجيني AA بين الأفراد الأصحاء. بالإضافة إلى ذلك، كانت مستويات IGF1 في المصل مرتفعة بشكل ملحوظ لدى المرضى (الوسيط: 110 بيكوغرام/مل) مقارنة بالأصحاء (الوسيط: 65 بيكوغرام/مل؛ $p < 0.001$). وبالمثل، كانت مستويات MIF أعلى لدى المرضى (85 بيكوغرام/مل مقابل 45 بيكوغرام/مل؛ $p < 0.001$) ، وكذلك تركيزات CXCL12 (140 بيكوغرام/مل مقابل 40 بيكوغرام/مل؛ $p < 0.001$). في المقابل، كانت مستويات IL−27 أقل لدى مرضى سرطان البروستات (40 بيكوغرام/مل) مقارنة بالأصحاء (65 بيكوغرام/مل؛ $p < 0.001$). أظهر تحليل منحنيات ROC أن كلًا من CXCL12 و MIF حققا دقة تشخيصية مثالية (AUC = 1.0) ، بينما أظهر IGF1 أداءً تشخيصيًا ممتازًا (AUC = 0.979) ، وحقق IL−27 أداءً جيدًا (AUC = 0.903). وقد ارتبط النمط الجيني GA للمتغير rs5742621 بشكل ملحوظ بارتفاع مستويات IGF1 وزيادة خطر الإصابة بسرطان البروستات، من خلال تعزيز تكاثر الخلايا، وتثبيط الاستماتة، وتسريع نمو الورم. يمكن اعتبار IGF1 والمؤشرات المناعية المرتبطة به مؤشرات واعدة للتشخيص وأهدافًا علاجية محتملة لسرطان البروستات.

1. Introduction

Prostate cancer (PCa) is the second most common malignancy in men worldwide, influenced by both genetic and environmental factors [1]. Global prevalence rates vary considerably due to alterable risk factors, such as age, race, family history, and genetics [2]. The etiology of PCa is believed to be influenced by the presence of various cytokines and hormones in the circulation, including insulin like growth factor 1 (IGF-1) and testosterone. Numerous single nucleotide polymorphisms (SNPs) associated with PCa are located within genes involved in cell cycle regulation, androgen metabolism, DNA repair, inflammation, and cytokine signaling. These SNPs contribute to individual variability in susceptibility to PCa [3].

IGF-1, also known as somatomedin C, is a hormone structurally similar to insulin and encoded by the *IGF1* gene. It promotes prostate cell proliferation and inhibits apoptosis,

thereby enhancing the viability of cancer cells. Additionally, IGF-1 strengthens androgen receptor signaling, an essential driver of PCa progression, and activates inflammatory and angiogenic pathways that facilitate tumor growth [4]. Several SNPs in the IGF-1 promoter region, such as rs35767 and rs5742621, have been correlated with increased PCa risk by raising serum levels of IGF-1. These impacts may arise from modified transcription factor binding affinities, chromatin remodeling, or epigenetic modifications [5]. The rs5742621 (A>G) variant, located in a regulatory region of the IGF-1 gene, may effect gene expression and serum IGF1 levels. This, in turn, can impair immune homeostasis and signaling, promot the excessive production of pro-inflammatory cytokines, and promote PCa progression [6].

Macrophage migration inhibitory factor (MIF) is a pro-inflammatory, pleiotropic cytokine produced by both malignant and stromal fibroblasts cells. It promots tumor cell proliferation, suppresses apoptosis, supports angiogenesis, and enhances extracellular matrix invasion, thus driving malignant growth and metastasis. Increased circulating levels of MIF have been identified in several cancer types, including bladder, lung, prostate, and breast cancer. In PCa, elevated MIF levels in serum and tumor tissues are linked to poor prognosis, reduced survival, greater tumor aggressiveness, and resistance to therapy [7].

Interleukin-27 (IL-27) is an anti-inflammatory mediator that signals through a heterodimeric receptor complex. It modulates immune responses by reducing Th17 cell differentiation, inducing regulatory T cells, and inhibiting excessive inflammation to prevent tissue damage [8]. Depending on the cancer type and immune context, IL-27 may exert both pro- and anti-tumor effects. In preclinical models, IL-27 has proved potent anti-tumor activity and is considered a promising therapeutic candidate [9]. Specifically, IL-27 inhibits PCa growth by disrupting the cell cycle, suppressing angiogenesis via downregulation of vascular endothelial growth factor (VEGF), and reducing the metastatic potential [10].CXC motif chemokine ligand 12 (CXCL12), also known as stromal cell-derived factor 1 (SDF-1), is a chemokine involved in immune cell recruitment, angiogenesis, hematopoiesis, and tumor progression [11]. CXCL12, through its association with the CXCR4 receptor, modulates the migration and survival of assorted normal and malignant cell types, including those of the breast, prostate, and brain. In PCa, CXCL12 promotes epithelial mesenchymal transition (EMT), resulting in reduced cell adhesion and enhanced motility, which facilitates local invasion and distant metastasis. Additionally, it activates cancer associated fibroblasts and contributes to the formation of a pro-tumorigenic microenvironment [12].

To better understand the immunogenetic landscape of PCa, this study investigates the correlation between the *IGF1* gene variant rs5742621 (A>G) and serum levels of IGF1 and other key cytokines, including MIF, IL-27, and CXCL12, in PCa patients compared to healthy controls.

2. Methodology

Patients and methods

A case-control study was conducted from March 2024 to July 2024, involving 204 patients with PCa and 196 healthy individuals. The median ages of the patient and control groups were 67 and 67.5 years, respectively (p= 0.344). Patients were referred to the Urology Units of Al Yarmouk Teaching Hospital and Martyr Dhari Al-Fayad Hospital for diagnosis and treatment, where a specialized urologist assessed the severity of the disease. All participants provided written informed consent. The study was approved by the Ethics Committee of the Iraqi Ministry of Health (Approval Reference: 494, dated March 6, 2024).

Prostate tumor collection, classification, and staging

Prostate tumor samples were obtained via transurethral resection of the prostate (TURP) or prostatectomy. According to WHO guidelines, tumors were assessed and classified by a

consultant pathologist. Tumor grade was determined using the Gleason scoring system, which classifies tumors into low- or high-grade categories. Tumor staging adhered to the TNM classification, which evaluates the extent of the primary tumor (T), regional lymph node involvement (N), and distant metastasis (M). *Blood sample collection*

Venous blood samples were collected from all participants. Two milliliters of blood were placed in EDTA tubes for the extraction of genomic DNA. The remaining blood was transferred to gel tubes and centrifuged at 5000 rpm for 15 minutes to separate the serum. The serum was used to measure levels of somatomedin C (IGF1), macrophage migration inhibitory factor (MIF), interleukin-27 (IL-27), and CXC motif chemokine ligand 12 (CXCL12).

DNA extraction and detection of rs5742621 variants using the tetra-ARMS technique

Genomic DNA was extracted from blood samples using the Wizard Genomic DNA Purification Kit (Geneaid, Korea), the concentration and purity of DNA were assessed via a nanodrop, and then stored at −20 °C. The rs5742621 SNP was genotyped using the tetra-primer amplification refractory mutation system-polymerase chain reaction (T-ARMS-PCR) method, as previously [13]. Primers were designed using an online T-ARMS PCR tool and validated using the Primer-BLAST tool from NCBI (https://www.ncbi.nlm.nih.gov/tools/primer-blast). Details of the T-ARMS-PCR primers and conditions are provided in Table 1. Primers were synthesized by Macrogen (Korea). The PCR products were electrophoresed on a 2% agarose gel and were observed under ultraviolet illumination.

Table 1: Primer sequence for the detection of rs5742621 variants

SNP/primer; 5′-3′rs5742621	Amplicon Size (bp)	Steps	Tm (°C)	Time (min.)	Cycle
IF CCCATAGGTTTTGAAAACAAAACCA IR GACAGGACAAAATAACCCTGAGGTATTTAC OF CAAATCAGTATGGCATTTCAGTTTGC OR ATCCTAGGCCTCTGAGATTCCTCAC	Allele A:235 Allele G:299 OP:479	Initial denaturation	95	5	1
		Denaturation	94	1	35
		Annealing	61	1	
		Extension	72	1	
		Final extension	72	7	1

SNP: Single nucleotide polymorphism; IF: Inner forward; IR: Inner reverse; OF: Outer forward; OR: Outer reverse; OP: Outer primer; PCR: Polymerase chain reaction.

Insulin growth factor 1 and cytokine immunoassays

Serum concentrations of somatomedin C (IGF1), MIF, IL-27, and CXCL12 were measured using enzyme-linked immunosorbent assay (ELISA) kits, following the manufacturer's instructions (Catalog Numbers: ab100545, ab100594, ab267812, and ab100637, respectively, Abcam, USA).

Statistical Analysis

Categorical variables were presented as frequencies and percentages. Differences between groups were assessed using a chi square test. The normality of continuous variables was evaluated using the Shapiro-Wilk test, which indicated a non-normal distribution. Therefore, these variables were reported as medians with interquartile ranges (IQR: 25th–75th percentiles). Group comparisons were performed using the Kruskal-Wallis test (for more than two groups) or the Mann-Whitney U test (for two groups). Receiver operating characteristic (ROC) curve analysis was conducted to determine the area under the curve (AUC). Correlations between cytokine levels were analyzed using Spearman's rank correlation coefficient. Hardy-Weinberg equilibrium (HWE) was tested using Pearson's Chi-square test.To assess the association between genetic variants and PCa risk, odds ratios (OR) with 95% confidence intervals (CI) were calculated. A p-value of <0.05 was considered statistically significant. For multiple comparisons, p-values were adjusted using the Bonferroni correction or Dunn's test, as appropriate. All statistical analyses were performed using GraphPad Prism

version 9.2.0 (GraphPad Software, Boston, MA, USA) and IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA). Power analysis and sample size estimation were carried out using G*Power version 3.1.9.7 [14].

3. Results

Baseline Features

Baseline characteristics of PCa patients and healthy controls, including age, WBC count, ESR, PSA levels, smoking status, family history, tumor stage, and grade, are presented in Table 2. The median age (IQR) did not differ significantly between the PCa group and the controls (67.5 [60.0–70.0] vs. 67.0 [58.0–69.0] years; p = 0.344). PCa patients showed significantly elevated WBC counts (8.9 [6.9–11.7] vs. 7.0 [6.8–7.9] × 10^9/L; p < 0.001), ESR (48.0 [39.0–58.0] vs. 17.0 [16.0–19.0] mm/h; p < 0.001), and PSA levels (17.0 [11.4–22.3] vs. 4.5 [4.0–6.2] ng/mL; p < 0.001) compared to healthy controls. Among the patients, 142 (69.6%) were smokers, while 62 (30.4%) were nonsmokers. In contrast, 64 (32.7%) of the controls were smokers, and 132 (67.3%) were nonsmokers. A family history of PCa or other cancers was reported in 134 (65.7%) patients. Histopathological assessment based on TNM staging revealed that 110 patients (53.9%) were in stage I–II, and 94 (46.1%) were in stage III–IV. Regarding tumor grade, 92 (45.1%) were classified as low-grade and 112 (54.9%) as high-grade. Although WBC counts in PCa patients remained within the normal range, elevated ESR values in patients and low ESR in healthy individuals (<20 mm/h) suggest the presence of inflammation in the patient group and its absence in controls.

Table 2: Baseline characteristics of patients with prostate cancer and healthy individuals.

Characteristics: median (IQR) or n (%)		PCa; n = 204	HC; n = 196	*p*-value
Age; years		67.5 (60.0-70.0)	67.0 (58.0-69.0)	0.344
WBC count; × 10^9/L		8.9 (6.9-11.7)	7.0 (6.8-7.9)	**< 0.001**
ESR; mm/h		48.0 (39.0-58.0)	17.0 (16.0-19.0)	**< 0.001**
PSA; ng/Ml		17.0 (11.4-22.3)	4.5 (4.0-6.2)	**< 0.001**
Cigarette-smoking	Yes	142 (69.6)	64 (32.7)	**< 0.001**
	No	62 (30.4)	132 (67.3)	
Family history	Yes	134 (65.7)	NA	
	No	70 (34.3)	NA	
Tumor stage	I-II	110 (53.9)	NA	
	III-IV	94 (46.1)	NA	
Tumor grade	Low	92(45.1)	NA	
	High	112 (54.9)	NA	

IQR: Interquartile range (25-75%); PCa: Prostate cancer; HC: Healthy controls; WBC: White blood cells; ESR: Erythrocyte sedimentation rate; PSA: Prostate-specific antigen; NA: Not applicable; *p*: Probability (significant *p*-value is indicated in bold). Significant differences between medians were assessed using the Mann-Whitney U test, and differences between percentages were assessed using the Pearson chi-square test.

Serum Levels of IGF1, IL-27, MIF, and CXCL12

Serum concentrations of IL-27, CXCL12, MIF, and IGF-1 differed significantly between PCa patients and healthy controls (HCs), as illustrated in Figure 1. IL-27 levels were significantly lower in PCa patients compared to HCs, with a median [IQR] of 40 [25–50] pg/mL

vs. 65 [60–70] pg/mL, respectively (***p < 0.001) (Figure 1A). Conversely, CXCL12 and MIF levels were significantly higher in patients with PCa. Median CXCL12 concentrations in PCa patients were about 140 [90–180] pg/mL, compared to 40 [35–45] pg/mL in HCs (***p < 0.001) (Figure 1B). Similarly, MIF levels were elevated in PCa patients, with median values of 85 [75–95] pg/mL, compared to 45 [40–50] pg/mL in HCs (***p < 0.001) (Figure 1C). Additionally, IGF1 levels were markedly increased in PCa patients compared to healthy individuals. The median serum IGF1 level was 110 pg/mL in the PCa group, significantly higher than 65 pg/mL in the HC group (**p < 0.001). These data are presented as medians with interquartile ranges (IQRs) (Figure 1D).

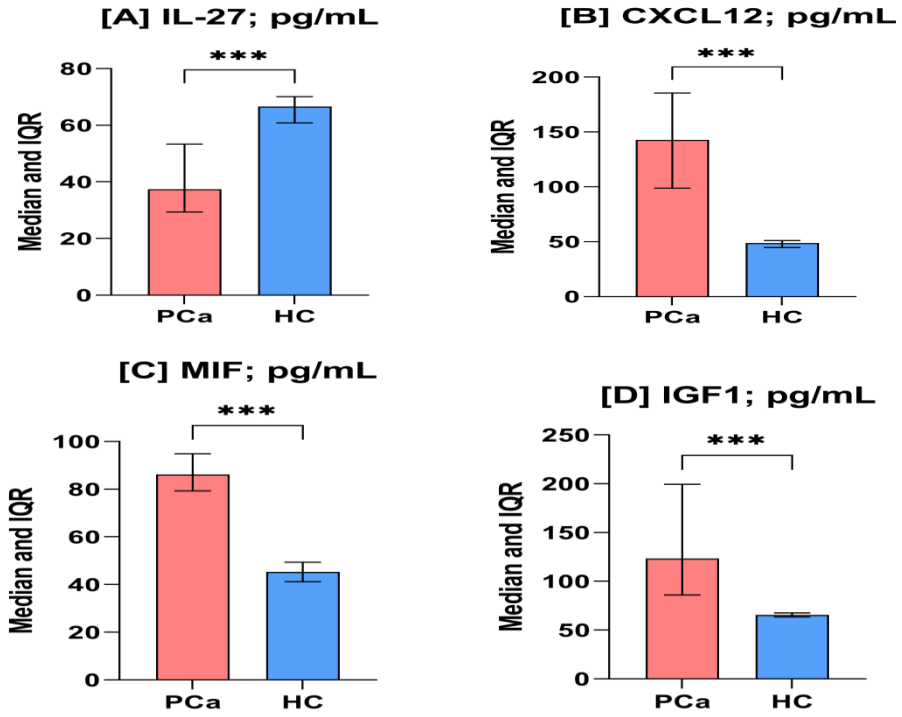

Figure 1: Column-bar plots of serum IL-27, CXCL12, MIF, and IGF1 levels [plots A, B, C, and D, respectively] in patients with PCa and HC. Columns indicate median. Bars indicate interquartile range (IQR: 25-75%). Significance was assessed using the Mann-Whitney U test (****p* < 0.001). PCa: Prostate cancer; HC: Healthy controls; IL-27: Interleukin-27; CXCL12: CXC motif chemokine ligand 12; MIF: Macrophage migration inhibitory; IGF1: Insulin-like growth factor 1.

Diagnostic performance of IL-27, CXCL12, MIF, and IGF-1 (ROC Analysis)

As shown in Figure 2, receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic utility of IL-27, CXCL12, MIF, and IGF-1 in distinguishing patients with PCa from HCs. IL-27demonstrated excellent diagnostic performance, with an area under the curve (AUC) of 0.903 and a statistically significant p-value (p < 0.001), indicating strong discriminatory power. Although not perfect, its high sensitivity and specificity indicate IL-27 is a dependable marker with significant potential for clinical applications. CXCL12 yielded an AUC of 1.0, demonstrating perfect diagnostic accuracy with 100% sensitivity and specificity. The result is statistically robust (p < 0.001) and confirms that CXCL12 is an ideal biomarker for differentiating patients with PCa from healthy individuals. Similarly, MIF achieved an AUC of 1.0, revealing perfect grading with 100% sensitivity and specificity, and highly significant statistical support (p < 0.001). These outcomess highlight MIF's strong potential as a standalone diagnostic biomarker. IGF1 revealed perfect diagnostic accuracy, with an AUC

of 0.979, sensitivity and specificity approaching 98%, and a highly significant p-value of < 0.001. While slightly lower than CXCL12 and MIF, IGF1 outperformed IL-27 and demonstrated excellent classification ability. It may serve effectively as a confirmatory or supplementary marker, especially when associated with IL-27 to enhance overall diagnostic sensitivity and specificity.

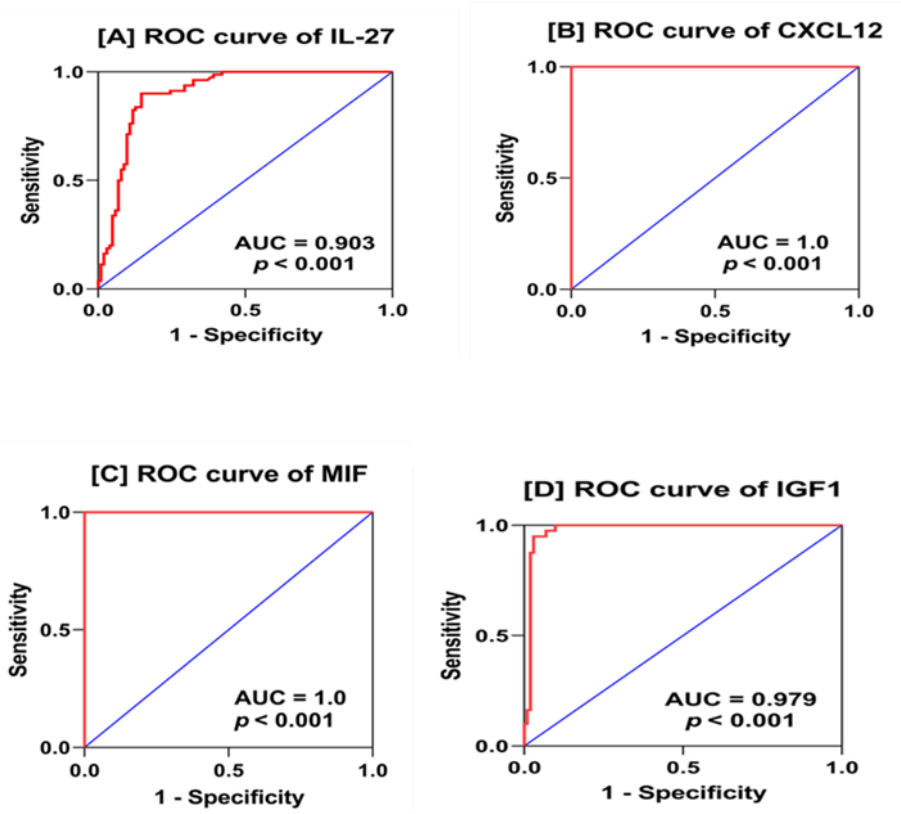

Figure 2: Receiver Operating Characteristic (ROC) curves for the diagnostic performance of IL-27 (A), CXCL12 (B), MIF (C), and IGF-1 (D). Area Under the Curve (AUC) values indicate high diagnostic accuracy for all biomarkers, with CXCL12 and MIF achieving perfect AUC scores of 1.0. Statistical significance for all curves is $p < 0.001$.

Association of serum cytokine levels with clinical and demographic variables

As presented in Table 3, serum concentrations of IL-27, CXCL12, MIF, and IGF-1 were analyzed in patients with PCa based on smoking status, family history of prostate cancer, tumor stage, and tumor grade. Smoking status: No statistically significant differences were observed in the serum levels of IL-27, CXCL12, MIF, or IGF-1 between smokers and nonsmokers ($p > 0.05$ for all comparisons). Family history: Patients with a family history of prostate cancer had significantly lower IL-27 levels ($p < 0.001$) and significantly higher levels of CXCL12 ($p < 0.001$), MIF ($p = 0.009$), and IGF1 ($p < 0.001$) compared to those without such a history. Tumor stage: Individuals with advanced-stage disease (stages III–IV) exhibited markedly lower IL-27 levels ($p < 0.001$) and significantly higher concentrations of CXCL12, MIF, and IGF-1 (all $p < 0.001$) compared to those with early-stage disease (stages I–II).Tumor grade: High-grade tumors were associated with significantly reduced IL-27 levels ($p < 0.001$) and elevated serum levels of CXCL12, MIF, and IGF-1 (all $p < 0.001$) when compared to low-grade tumors. These findings collectively suggest a consistent pattern wherein IL-27 levels decline, and CXCL12,

MIF, and IGF1 levels increase with more aggressive or advanced tumor features, indicating their potential utility as biomarkers for disease severity and progression in prostate cancer.

Table 3: Serum levels of IL-27, CXCL12, MIF, and IGF1 according to characteristics of patients with prostate cancer.

Characteristics		Median (IQR; 25-75%); pg/ml			
		IL-27	CXCL12	MIF	IGF1
Cigarette-smoking	Yes	36.1 (29.3-54.1)	139.1 (98.6-184.7)	87.5 (81.3-95.4)	155.2 (86.8-199.4)
	No	42.6 (31.1-49.4)	146.4 (99.5-198.5)	85.9 (74.7-92.1)	105.3 (85.5-209.5)
	p-value	0.907	0.965	0.259	0.887
Family history	Yes	32.6 (28.4-48.2)	167.5 (100.8-199.3)	89.2 (82.1-98.4)	184.5 (95.4-209.5)
	No	52.3 (42.6-56.2)	104.2 (94.5-116.4)	85.9 (77.1-87.5)	91.5 (79.5-118.7)
	p-value	**< 0.001**	**< 0.001**	**0.009**	**< 0.001**
Tumor stage	I-II	52.3 (46.3-58.4)	100.2 (94.5-113.8)	81.3 (74.7-87.1)	89.2 (80.3-105.3)
	III-IV	29.6 (25.7-33.8)	184.7 (167.4-203.5)	94.7 (87.2-109.6)	195.5 (182.2-224.6)
	p-value	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Tumor grade	Low	53.5 (48.6-60.6)	98.7 (93.8-104.3)	79.3 (72.1-85.9)	85.5 (79.3-92.1)
	High	30.0 (26.2-33.7)	181.6 (162.5-201.4)	93.7 (86.6-106.5)	195.0 (183.3-218.7)
	p-value	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**

IQR: Interquartile range; IL-27: Interleukin-27; CXCL12: CXC motif chemokine ligand 12; MIF: Macrophage migration Inhibitory factor; IGF1: Insulin-like growth factor 1; *p*: Probability (significant *p*-value is indicated in bold). Significant differences between medians were assessed using the Mann-Whitney U test.

rs5742621 variant in the IGF-1 promoter

According to the results of Table 4, the frequency of the G allele was significantly higher in PCa patients (35.3%) compared to HCs (18.9%), corresponding to an increased risk of PCa (OR = 2.36, 95% CI: 1.45–3.85, p = 0.001). In genotype analysis, the heterozygous AG genotype was more frequent in PCa patients (45.1%) than in controls (27.5%). It was associated with a significantly increased risk of PCa (OR = 2.63, 95% CI: 1.38–4.99, p = 0.004) compared to the AA genotype. Similarly, the homozygous GG genotype showed an even higher risk (OR = 4.08, 95% CI: 1.25–13.28, p = 0.018), although it was less frequent (12.7% in PCa vs. 5.0% in controls). The genotype distributions were in Hardy-Weinberg equilibrium (HWE) among both PCa cases (p = 0.898) and controls (p = 0.383). These results suggest that the rs5742621 G allele and the AG and GG genotypes are significant genetic risk factors for prostate cancer, with the potential for the rs5742621 variant to serve as a marker for assessing individual susceptibility to PCa.

Table 4: Association analysis of rs5742621 (*IGF1* promoter variant) with the risk of PCa.

rs5742621 allele/genotype	PCa; n = 204		HC; n = 196		OR (95% CI)	*p*-value
	N	%	N	%		
A	264	64.7	318	81.1	Reference; 1.0	
G	144	35.3	74	18.9	2.36 (1.45- 3.85)	**0.001**
AA	86	42.2	132	67.5	Reference; 1.0	
GA	92	45.1	54	27.5	2.63 (1.38-4.99)	**0.004**
GG	26	12.7	10	5.0	4.08 (1.25-13.28)	**0.018**
p-HWE	0.898		0.383			

PCa: Prostate cancer; HC: Healthy controls; OR: Odds ratio; CI: Confidence interval; HWE: Hardy-Weinberg equilibrium; *p*: Two-tailed probability (significant *p*-value is indicated in bold).

Association between rs5742621 genotypes and serum IGF-1 levels

The analysis of serum IGF-1 levels according to rs5742621 genotypes, summarized in Table 5, revealed a significant association between the presence of the G allele and increased IGF-1 levels across all participants, as well as within specific groups. Among all participants, IGF-1 levels progressively increased with the presence of the G allele: individuals with the AA genotype had a median IGF-1 level of 67.4 pg/mL (IQR: 64.3–84.9), compared to 93.9 pg/mL (IQR: 70.5–203.8) for GA genotypes and 184.5 pg/mL (IQR: 98.7–203.8) for GG genotypes ($p < 0.001$).In the PCa group, IGF-1 levels were markedly higher across genotypes, with median values of 86.2 pg/mL (AA), 187.4 pg/mL (GA), and 195.5 pg/mL (GG) ($p < 0.001$).In contrast, among healthy controls (HC), IGF-1 levels remained relatively low and stable, with median levels of 65.2 pg/mL (AA), 67.6 pg/mL (GA), and 67.5 pg/mL (GG). Despite these low levels, a statistically significant difference was observed ($p = 0.002$). These results suggest that the rs5742621 G allele is strongly associated with elevated IGF-1 levels, particularly in PCa patients, highlighting its potential role in the progression of prostate cancer.

Table 5: Serum levels of IGF1 according to rs5742621 genotypes in PCa patients and healthy controls.

rs5742621 genotypes	IGF1 level; median (IQR: 25-75); pg/ml		
	All participants; n = 400	PCa; n = 204	HC; n =196
AA	67.4 (64.3-84.9)	86.2 (79.1-172.1)	65.2 (61.5-67.3)
GA	93.9 (70.5-203.8)	187.4 (92.3-219.0)	67.6 (63.5-70.5)
GG	184.5 (98.7-203.8)	195.5 (182.2-211.5)	67.5 (67.4-69.0)
p-value	**< 0.001**	**< 0.001**	**0.002**

IGF1: Insulin-like growth factor 1; PCa: Prostate cancer; HC: Healthy controls; IQR: Interquartile range; *p*: Probability (significant *p*-value is indicated in bold). Significant differences between medians were assessed using the Kruskal-Wallis test.

Results in Table 6 showed the distribution of *IGF-1* rs5742621 genotypes with PCa stage and grade. A significant association was observed between genotype and cancer stage ($p < 0.001$). The AA genotype was predominantly found in patients with early-stage disease (stages I–II; 79.1%), whereas the GA (63.0%) and GG (69.2%) genotypes were more frequent in patients with advanced-stage disease (stages III–IV). Similarly, genotype distribution correlated strongly with tumor grade ($p < 0.001$). The AA genotype was predominantly associated with low-grade tumors (72.1%), while the GA and GG genotypes were significantly more common

in high-grade tumors (69.6% and 92.3%, respectively). These results suggest that the presence of the G allele is linked to more aggressive forms of prostate cancer.

Table 6: *IGF-1* rs5742621 genotypes classified according to stage and grade of PCa.

Stage/grade		*IGF-1* rs5742621 genotypes; n=204						*p*-value
		AA; n = 86		AG; n = 92		GG; n = 26		
		N	%	N	%	N	%	
Stage	I-II	68	79.1	34	37.0	8	30.8	**< 0.001**
	III-IV	18	20.9	58	63.0	18	69.2	
Grade	Low	62	72.1	28	30.4	2	7.7	**< 0.001**
	High	24	27.9	64	69.6	24	92.3	

IGF1: Insulin-like growth factor 1; *p*: Two-tailed probability (significant *p*-value is indicated in bold)

Correlation analysis of biomarkers

In Figure 3, the correlation matrix presented in this study evaluates the relationships between PSA, IL-27, CXCL12, MIF, and IGF-1, revealing key interactions: PSA shows strong positive correlations with CXCL12 (0.685), MIF (0.622), and IGF-1 (0.704), indicating a close relationship between PSA and these biomarkers. It also demonstrates a strong negative correlation with IL-27 (-0.698), suggesting an inverse relationship.IL-27 exhibits strong negative correlations with CXCL12 (-0.680) and IGF-1 (-0.725), as well as a moderate negative correlation with MIF (-0.550). This suggests that IL-27 may act as an inhibitory factor relative to these biomarkers, potentially playing a suppressive role in the associated pathways. CXCL12 exhibits a strong positive correlation with IGF-1 (0.706), suggesting that these two biomarkers may be involved in complementary biological processes. Additionally, CXCL12 and MIF demonstrate a moderate positive correlation (0.553), confirming their potential interconnection in the inflammatory or tumor related microenvironment. MIF shows a moderate positive correlation with IGF-1 (0.597), further suggesting a relationship between these inflammatory markers and the IGF-1 pathway. IGF-1 demonstrates strong positive correlations with PSA (0.704) and CXCL12 (0.706), as well as a moderate positive correlation with MIF (0.597), supporting its role in a shared pathway with these biomarkers. PSA, CXCL12, MIF, and IGF1 generally exhibit positive correlations, revealing potential synergistic roles in prostate cancer biology, possibly reflecting complementary or related pathways involved in disease advancement IL-27 demonstrates strong negative correlations with all other immune markers, emphasizing its potential as an inhibitory factor, especially in relation to IGF-1 (the strongest negative correlation). This suggests that IL-27 may serve as an anti-inflammatory or suppressive agent relative to the other markers. These findings underline the complex interplay between these biomarkers and their potential utility in understanding prostate cancer progression and therapeutic targeting.

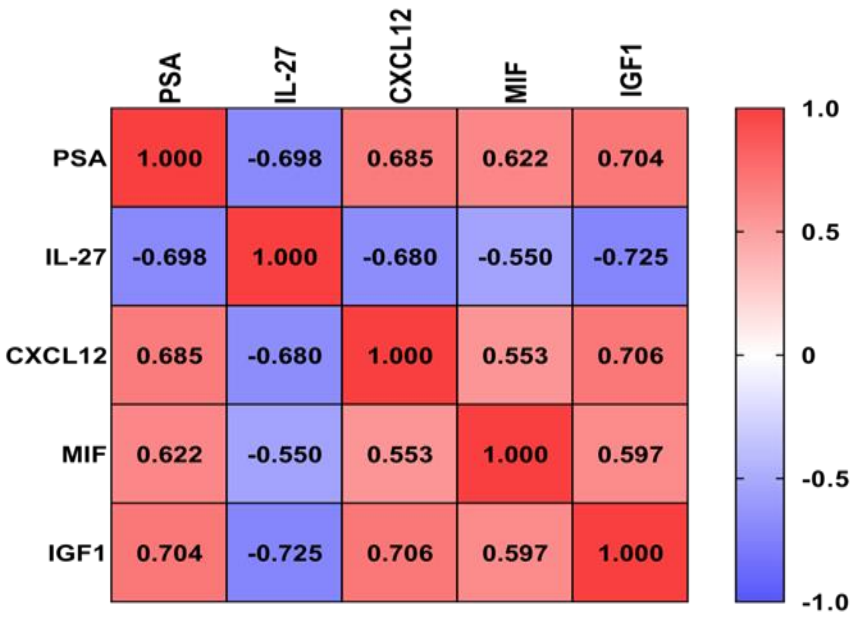

Figure 3: A heat-map matrix of correlation analysis between PSA (prostate-specific antigen), IL-27 (interleukin-27), CXCL12 (CXC motif chemokine ligand 12), MIF (macrophage migration inhibitory factor), and IGF1 (insulin-like growth factor 1) in patients with prostate cancer. The values inside the boxes indicate the correlation coefficient (r_s). Spearman's rank correlation analysis was performed to calculate the r_s between each pair of variables. Positive correlation is highlighted in red, while negative correlation is highlighted in blue. The darker color is the higher value of the r_s. All variables show a significant positive or negative correlation with a two-tailed probability < 0.001.

4. Discussion

Age, hormonal profile, smoking, and recurrent urinary tract infections are well-known risk factors that contribute to the pathophysiology of common prostate diseases, including PCa. [15]. The baseline data revealed several important differences between PCa patients and HCs. There was no significant age difference between patients with PCa and healthy controls. This is expected, as both groups were likely age-matched for study design purposes. PCa incidence increases with age, particularly after 50 years, but the lack of a significant age difference ensures that observed results in biomarkers are not confounded by age. Patients with PCa had significantly higher WBC counts and ESR compared to healthy controls. While the WBC count remained within normal limits, its relative elevation may reflect systemic immune activation or chronic inflammation often associated with malignancy. Elevated ESR is a nonspecific marker of inflammation and has been linked to poor prognosis in various cancers, including PCa. Chronic inflammation can promote tumorigenesis by inducing genetic mutations, promoting angiogenesis, and suppressing anti-tumor immunity [16]. PSA levels were significantly higher in PCa patients, which were produced by normal and malignant prostate cells. In cancer, particularly high-grade tumors, integrity of the basement membrane is lost, allowing more PSA to leak into the bloodstream only, aggressive tumors produce more PSA overall due to rapid cell turnover, and tumor invasion into surrounding tissues further elevates serum PSA levels [17]. Thus, PSA is routinely used as a biomarker for screening, diagnosis, and monitoring of PCa progression and treatment response. A significantly higher proportion of PCa patients were smokers compared to healthy controls (69.6% vs. 32.7%). Smoking has been associated with increased prostate cancer risk and aggressiveness, possibly due to carcinogens that promote DNA damage, hormonal alterations, and immune suppression. Smoking is also known to exacerbate systemic inflammation, which may contribute to the

elevated ESR and WBC levels seen in patients. Over 65% of PCa patients reported a family history of prostate or other cancers, emphasizing the strong hereditary component of prostate cancer. Men with a first-degree relative (father or brother) diagnosed with PCa have a 2–3 times higher risk of developing the disease. The risk increases further with multiple affected relatives or earlier age at diagnosis, suggesting a strong familial component [18]. Genetic predisposition, particularly mutations in BRCA1/2, HOXB13, and other susceptibility genes, significantly elevates the risk of developing PCa. Epidemiological studies estimate that hereditary factors account for approximately 40–50% of PCa risk, making it one of the most heritable solid tumors. Several inherited mutations have been linked to an increased susceptibility to PCa and a more aggressive disease. Beyond high-penetrance mutations, numerous SNPs identified through genome-wide association studies (GWAS) contribute to PCa risk. While each SNP has a small effect size, combined polygenic risk scores (PRS) can effectively stratify men by lifetime risk and may enhance personalized screening strategies [19].

The distribution of tumor stages (53.9% in stages I–II and 46.1% in stages III–IV) and grades (55.9% high grade) indicates that a substantial proportion of patients had advanced or aggressive disease. This distribution is consistent with the observed elevation in inflammatory markers and PSA, which are known to correlate with tumor burden and progression. Advanced-stage and high-grade prostate cancers involve larger tumor masses and/or invasion beyond the prostate gland. As tumor cells proliferate and invade tissues, they disrupt the normal architecture of the prostate [20]. The elevation of inflammatory markers and PSA levels in patients with advanced-stage and high-grade prostate cancer reflects increased tumor burden and progression. As tumors grow and invade surrounding tissues, they trigger inflammation, immune responses, and cytokine release, creating a tumor-promoting microenvironment. This environment supports angiogenesis, immune evasion, and further tumor growth, contributing to the aggressive nature of the disease [21].

The present findings demonstrate significant alterations in the serum levels of IL-27, CXCL12, MIF, and IGF-1 in PCa patients compared to HCs, suggesting their involvement in PCa pathophysiologyIt is noteworthy that both CXCL12 and MIF achieved AUC values of 1.0 in ROC curve analysis, corresponding to 100% sensitivity and specificity. While these findings reflect ideal diagnostic separation between prostate cancer patients and healthy controls, such perfect discrimination is exceedingly rare in biomedical research. Typically, biological variability among individuals results in some overlap between groups, making an AUC of 1.0 uncommon. The absence of overlap observed in this study may be attributed to sample characteristics, strict inclusion criteria, or technical consistency in biomarker measurement. Nonetheless, these results should be interpreted with caution. To establish the true diagnostic utility of CXCL12 and MIF, further research involving larger and more diverse populations is essential. Independent validation in multi-center cohorts would help confirm the reproducibility of these findings and assess their robustness as clinical diagnostic markers for PCa. The decreased serum levels of IL-27 in patients may be attributed to several biological and pathological mechanisms, primarily related to immune evasion and tumor-driven immunosuppression. Prostate tumors can create an immunosuppressive microenvironment that inhibits the expression and release of cytokines, such as IL-27. Tumor-associated factors (e.g., TGF-β, IL-10, and regulatory T cells) can suppress antigen-presenting cells (APCs) such as dendritic cells and macrophages, which are major sources of IL-27 [22]. Cancer progression often involves a shift from a Th1-type immune response, which supports IL-27 expression, to a Th2 or Treg-dominated response, which reduces IL-27 production and promotes immune tolerance. Tumor-derived factors can cause epigenetic suppression of genes involved in IL-27

production or interfere with upstream signaling pathways. It's also possible that local production of IL-27 within the tumor is followed by rapid uptake by immune or tumor cells, leading to reduced systemic (serum) levels despite localized activity [23]
Elevated serum levels of MIF are commonly observed in PCa patients and are associated with tumor progression, immune modulation, and inflammation. The increase in MIF can be attributed to several mechanisms. PCa cells themselves overproduce MIF, which contributes to tumor growth and metastasis. As a survival and proliferative factor that supports cancer cell viability under stress conditions, such as hypoxia or nutrient deprivation, it enhances NF-κB and MAPK signaling, which are crucial for tumor survival, angiogenesis, and immune evasion [24]. In addition, MIF is known to antagonize p53, a critical tumor suppressor involved in DNA damage and the accumulation of genetic mutations. Hypoxia can stimulate MIF expression via hypoxia-inducible factors (HIFs), which in turn enhance angiogenesis by upregulating VEGF and other angiogenic factors [25].

Elevated CXCL12 levels in PCa patients are linked to several tumor-promoting processes. Both PCa cells and tumor-associated stromal cells, especially cancer-associated fibroblasts, overexpress CXCL12, which creates a chemokine gradient that supports cancer cell survival, migration, and invasion. PCa cells express CXCR4 and are attracted to CXCL12-rich organs, such as bone, a common site of metastasis in PCa. Hypoxic conditions in the tumor microenvironment upregulate CXCL12 via HIF-1α, which enhances angiogenesis, tumor survival, and resistance to therapy. Clinical studies have shown that higher CXCL12 levels correlate with aggressive PCa phenotypes, a poor prognosis, and an increased risk of recurrence [26, 27].
IGF-1 is a mitogen for prostate epithelial cells; elevated serum levels of it as a predictor of PCa incidence may have implications for risk reduction and treatment. In the USA, the risk of PCa has a strong, positive association with serum levels of IGF-1, especially among older men, and no significant difference is observed for high-grade/stage and low-grade/stage cancers [28]. IGF-1 is primarily produced in the liver under the control of growth hormone (GH). Some PCa-associated systemic factors may stimulate hepatic IGF-1 production. The most vital parameter that is affected by the expression level of IGF-1 is the dysregulation of the IGF-1/IGF-1 receptor (IGF1R) signaling pathway. Can enhance the activation of downstream pathways, such as the PI3K/Akt and MAPK, which promote cell survival and proliferation. PCa cells and the tumor microenvironment can produce IGF-1 autocrinally or paracrinally, which may lead to increased levels in circulation. IGF-1 is normally regulated by IGF-binding proteins (IGFBPs), decreased levels of these proteins can increase free, bioactive IGF-1, which is more likely to exert tumor-promoting effects [29].

Analysis of the rs5742621 polymorphism in the *IGF-1* promoter revealed a significant association with PCa susceptibility. The observed associations between the G allele and increased PCa risk, as well as the elevated risks conferred by GA and GG genotypes, can be explained by several potential biological and genetic factors: The G allele may alter the function or expression of the gene it resides in (or nearby), potentially affecting cellular pathways relevant to tumor development. Because polymorphism occurs in a regulatory region or causes a coding change, it may lead to increased expression of an oncogene, decreased expression of a tumor suppressor gene or altered activity of proteins involved in cell proliferation, apoptosis, or DNA repair [30]. The G allele may not be the direct cause of increased PCa risk, but it could be in linkage disequilibrium with another, functionally relevant variant that directly contributes to tumor development. The increasing odds ratios seen from GA to GG genotypes (OR = 2.63 to 4.08) suggest a dose-dependent effect, where the presence of one or two G alleles proportionally increases susceptibility to PCa. This supports the

hypothesis that the allele contributes functionally to disease risk. If the gene affected by the polymorphism is involved in inflammatory pathways, androgen metabolism, or immune regulation, the G allele may contribute to a microenvironment that is conducive to tumor initiation and progression [31].

Our analysis demonstrated a significant association between the rs5742621 G allele and elevated serum IGF-1 levels, with a clear genotype-dose effect observed across all participants. Individuals carrying the GG genotype exhibited the highest median IGF-1 concentrations, followed by the GA and AA genotypes, indicating a potential functional impact of this SNP on IGF-1 expression. rs5742621 is located in the promoter region of the *IGF-1* gene, which controls how much IGF-1 is transcribed. A nucleotide change (from A to G) in this regulatory region can alter the binding affinity of transcription factors, potentially enhance promoter activity and lead to increased transcription of IGF1 mRNA [32,33]. Tumor-related factors, such as androgen receptor signaling amplify the transcriptional effect of the G allele and epigenetic or microenvironmental changes in cancer that make the promoter more responsive to this variant. Potential interaction with oncogenic pathways that further elevate IGF-1 in the presence of the G allele [34,35]. Overall, the observed correlation patterns imply that PSA, CXCL12, MIF, and IGF-1 may participate in shared oncogenic pathways, whereas IL-27 may function as an inhibitory factor. The strongest positive correlation (CXCL12–IGF-1) and strongest negative correlation (IL-27–IGF-1) further highlight the potential central role of IGF-1 in mediating both pro- and anti-tumorigenic signals depending on its regulatory context.

Limitations, although the inclusion of 204 PCa patients and 196 HCs provides a reasonably large sample size, it may still fall short in representing the full diversity of the population, particularly in terms of ethnicity, age distribution, and regional variation. The investigation was limited to the rs5742621 variant in the *IGF-1* promoter, without examining additional genetic polymorphisms or mutations that may also influence PCa susceptibility and biomarker expression. Expanding the genetic analysis could yield a more comprehensive understanding of the molecular landscape underlying the disease. Furthermore, while IL-27, CXCL12, MIF, and IGF-1 were significantly associated with PCa in this study, their disease specificity remains unclear. These biomarkers may also be elevated in other malignancies or inflammatory conditions, warranting further comparative analyses to establish their uniqueness to prostate cancer. Additionally, data on family history were not collected for the control group, limiting the ability to adjust for hereditary risk factors and potentially introducing bias in assessing the true genetic contribution to prostate cancer susceptibility.

5. Conclusion

The *IGF-1* gene plays a crucial role in cellular growth, differentiation, and apoptosis. Variations in this gene can alter circulating levels of IGF-1, thereby influencing cancer risk. Heterozygous GA genotype also correlates with more advanced disease stages and higher tumor grades, suggesting that the G allele could serve as a genetic risk factor for prostate cancer. PCa patients exhibited significantly lower IL-27 and significantly higher CXCL12, MIF, and IGF-1 levels compared to HCs. These biomarkers show strong correlations with each other. The findings suggest that biomarkers, such as IL-27, CXCL12, MIF, and the *IGF-1* promoter variant rs5742621, could be incorporated into diagnostic and prognostic models to enhance the early detection and risk stratification of prostate cancer.

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Conflict of Interest

The authors declare that there are no commercial or financial relationships that could be construed as a potential conflict of interest with respect to the research.

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