ORIGINAL ARTICLE

UDC: 616.65-006-037
DOI: https://doi.org/10.2298/VSP250315072V

Peritumoral infiltration of CD3 lymphocytes makes a difference in prostate cancer

Peritumorska infiltracija CD3 limfocita pravi razliku kod karcinoma prostate

Milka Vještica*†, Sandra Trivunić-Dajko‡, Božana Babić†§, Saša Jungić *†, Jelena Berendika*†

University Clinical Center of the Republic of Srpska, *Clinic for Oncology, §Department of Pathology, Banja Luka, Republic of Srpska, Bosnia and Herzegovina; †University of Banja Luka, Faculty of Medicine, Banja Luka, Republic of Srpska, Bosnia and Herzegovina; ‡University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia

Abstract

Background/Aim. Prostate cancer (PC) is the second most common malignancy in men, and the fifth leading cause of cancer death. The most important prognostic parameters are tumor, node, and metastasis (TNM) status, initial prostate-specific antigen (PSA), Gleason score, and grade group. However, these parameters are insufficient for accurate prediction of the course of the disease. The aim of this study was to examine the prognostic and predictive value of $CD3^+$ T lymphocyte infiltration, including the $CD8^+$ T-cell subset, in PC tissue across various disease stages. **Methods.** A prospective cohort study included 90 newly diagnosed patients with PC, divided into three groups [30 with medium/high-risk localized disease (Group A), 30 with locally advanced disease (Group B), and 30 with metastatic disease (Group C)]. The patients were followed for 120 months (10 years). The difference in the density of $CD3^+$ and $CD8^+$ T lymphocytes was analyzed in the tumor tissue (TT) and at the invasive margin (IM). The influence of infiltration of $CD3^+$ T and $CD8^+$ T lymphocytes on progression-free survival (PFS) and overall survival (OS) in patients with PC was analyzed. Lymphocytic infiltration was quantified at the area of greatest density using an optical Leica microscope at ×200 magnification and photographed with a Leica camera. The field of view was 0.226377 mm^2. Lymphocytes were then manually counted with the aid of commercially available software Aperio 12.0. Correlations with clinical parameters, PFS, and OS were analyzed using receiver operating characteristic (ROC) curves, Kaplan-Meier survival analysis, and correlation coefficients. **Results.** High peritumoral infiltration of $CD3^+$ lymphocytes was an independent predictor of shorter PFS and OS (log-rank $p < 0.001$). $CD3^+$ IM infiltration correlated significantly with initial PSA, nadir PSA, and perineural invasion ($p < 0.05$), while there was no correlation with Gleason score, International Society of Urological Pathology (ISUP) grade, or age. Thresholds of $CD3^+$ IM predictive of cancer-specific mortality [area under the curve (AUC) = 0.681, $p = 0.0017$], OS (AUC = 0.634, $p = 0.0173$), and PFS (AUC = 0.657, $p = 0.0062$) were identified using ROC analysis. No significant prognostic value was found for $CD3^+$ T lymphocyte infiltration in the TT, nor for $CD8^+$ T lymphocyte infiltration in either TT or at the IM. **Conclusion.** $CD3^+$ T lymphocyte infiltration at IM is a significant prognostic and predictive biomarker in PC, indicating tumor aggressiveness and potential influence on treatment response. These findings support the integration of immune profiling into routine histopathological evaluation to refine risk stratification and guide personalized treatment strategies in patients with PC.

Key words:
biomarkers; progression-free survival; prostatic neoplasms; survival; t-lymphocytes.

Apstrakt

Uvod/Cilj. Karcinom prostate (KP) je drugi po učestalosti karcinom kod muškaraca i peti vodeći uzrok smrtnosti od karcinoma. Najvažniji prognostički parametri su stadijum tumora, limfnih čvorova i metastaza (*tumor, node, metastasis* – TNM), početni nivo prostata-specifičnog antigena (PSA), Gleason-ov skor i gradus grupa tumora. Međutim, ovi parametri su nedovoljni za precizno predviđanje toka bolesti. Cilj rada bio je da se ispita prognostička i prediktivna vrednost infiltracije $CD3^+$ T limfocita, uključujući $CD8^+$ T subpopulaciju, u tkivu KP kroz različite stadijume bolesti. **Metode.** Prospektivna kohortna studija obuhvatila je 90 novodijagnostikovanih obolelih od KP podeljenih u tri grupe [30 sa lokalizovanom bolešću srednjeg/visokog rizika (Grupa A), 30 sa lokalno uznapredovalom bolešću (Grupa B) i 30 sa metastatskom bolešću (Grupa C)]. Bolesnici su praćeni 120 meseci (10 godina). Razlika u gustini $CD3^+$ i $CD8^+$ T limfocita analizirana je u tumorskom tkivu (TT) i na invazivnoj margini (IM). Analiziran je uticaj infiltracije $CD3^+$

Correspondence to: MilkaVještica, University Clinical Center of the Republic of Srpska, Clinic for Oncology, St. 12 beba, 78 000 Banja Luka, Republic of Srpska, Bosnia and Herzegovina. E-mail: milka.vjestica@kc-bl.com

T i CD8+ T limfocita na preživljavanje bez progresije bolesti (*progression-free survival* – PFS) i ukupno preživljavanje (*overall survival* – OS) obolelih od KP. Infiltracija limfocita kvantifikovana je na području najveće gustine korišćenjem optičkog Leica mikroskopa pri uvećanju od ×200 i fotografisana Leica kamerom. Veličina vidnog polja iznosila je 0,226377 mm². Limfociti su zatim ručno prebrojani uz pomoć komercijalno dostupnog softvera Aperio 12.0. Korelacije sa kliničkim parametrima, PFS i OS analizirane su korišćenjem *receiver operating characteristic* – ROC krive, Kaplan-Meier analize preživljavanja i koeficijenata korelacije. **Rezultati.** Visoka peritumorska infiltracija CD3+ limfocita bila je nezavisni prediktor kraćeg PFS i OS (*log-rank* $p < 0,001$). Infiltracija CD3+ T limfocita u IM značajno je korelirala sa početnim vrednostima PSA, najnižim vrednostima (nadir) PSA i perineuralnom invazijom ($p < 0,05$), dok nije bilo korelacije sa Gleasonovim skorom, gradusom *International Society of Urological Pathology* – ISUP ili starošću bolesnika. Granične vrednosti CD3+ IM koje predviđaju smrtnost zbog karcinoma [*area under the curve* (AUC) = 0,681, $p = 0,0017$], OS (AUC = 0,634, $p = 0,0173$) i PFS (AUC = 0,657, $p = 0,0062$) identifikovane su ROC analizom. Nije pronađena značajna prognostička vrednost za infiltraciju CD3+ T limfocita unutar TT, niti za infiltraciju CD8+ T limfocita u TT ni na IM. **Zaključak.** Infiltracija CD3+ T limfocita na IM predstavlja značajan prognostički i prediktivni biomarker kod KP, koji odražava agresivnost tumora i potencijalno utiče na terapsijki odgovor. Ovi nalazi podržavaju integraciju imunskog profilisanja u rutinsku histopatološku evaluaciju u cilju poboljšanja procene rizika i vođenja personalizovanih terapijskih strategija kod obolelih od KP.

Ključne reči:
biomarkeri; preživljavanje, bez progresije; prostata, neoplazme; preživljavanje; limfociti t.

Introduction

Prostate cancer (PC) is the second most common cancer among men worldwide and the fifth leading cause of cancer death [1]. Patients with PC can present in very different forms of the disease, ranging from localized hormone-sensitive forms to metastatic castration-resistant forms. The most important prognostic parameters include tumor, node, and metastasis (TNM) status, initial prostate-specific antigen (PSA) level, Gleason score (GS), and grade group. However, these parameters are insufficient for an accurate prediction of the course of the disease. New biomarkers are needed to improve risk stratification and enable more personalized treatment [2]. The role of the local immune response is widely discussed. In recent years, particular emphasis has been placed on the tumor microenvironment as a significant factor that can either inhibit or promote tumor growth. Tumor-infiltrating lymphocytes (TILs), arising as part of the inflammatory response to malignant cells, represent an important component of the antitumor defense. Their location, function, and abundance provide valuable insights into the nature and strength of the immune response against cancer [3]. For a long time, PC was considered an immunologically "cold" cancer. However, new research challenges this view, demonstrating that PC has a highly heterogeneous immune environment, with the immune landscape playing a significant role in determining the disease's prognosis. The immune system can potentially play dual roles, either promoting or suppressing tumor progression and metastasis [4]. Additionally, the localization of TILs at the invasive margin (IM) and within the tumor tissue (TT) plays an important role in tumor biology and patient outcomes [5]. During carcinogenesis, immune cells, including T and B lymphocytes, accumulate in prostate tissue [6]. T lymphocytes, all of which express CD3, are essential for immune surveillance, with CD8+ T cells playing a key role in antitumor immunity.

The aim of this study was to examine the prognostic and predictive value of CD3+ and CD8+ T lymphocyte infiltration in PC tissue across various disease stages by analyzing differences in their density.

Methods

The study was initiated as a prospective cohort study, including 90 patients newly diagnosed with adenocarcinoma of the prostate [30 with medium/high-risk localized disease (Group A), 30 with locally advanced disease (Group B), and 30 with metastatic disease (Group C)], between January 1, 2013, and January 1, 2015. The patients were followed for disease progression and survival over the subsequent 120 months (10 years). Demographic and clinical data were obtained from medical records, while histopathological data for all cases were reviewed and registered by two experienced pathologists (BB and MB). The tumors were graded according to the International Society of Urological Pathology (ISUP) grading system and GS. Patients were classified into risk groups according to the European Association of Urology recommendations [7]. Intermediate-risk patients were defined as those with tumor category cT2b or GS 7 (ISUP grade 2/3), or PSA levels between 10 ng/mL and 20 ng/mL. High-risk patients were defined as those with tumor category cT2c or GS ≥ 8 (ISUP grade 4/5) or PSA levels >10–20 ng/mL. Locally advanced patients are defined as those with tumor category cT3-T4 or N1, regardless of GS, ISUP grade, or PSA level [7]. The patients with metastatic disease had one or more metastases confirmed by one of the imaging methods (computed tomography, magnetic resonance, positron emission tomography/computed tomography, etc.).

Following the diagnostic workup, the disease stage was determined in each patient. All cases were subsequently reviewed by a multidisciplinary oncology board, which developed individualized treatment plans.

Initial therapy for all patients consisted of androgen deprivation therapy (ADT), achieved either through the administration of luteinizing hormone-releasing hormone analogs and antiandrogens (bicalutamide) or *via* bilateral

orchiectomy. In patients with localized and locally advanced PC, ADT was administered for a duration of two to three years. Patients with metastatic disease received continuous ADT for the remainder of their clinical course.

After six months of ADT, patients with localized and locally advanced disease were treated with definitive external beam radiotherapy to the prostate. In patients with metastatic disease, palliative radiotherapy was administered to bone lesions as clinically indicated.

Of the total number of patients, 95.6% underwent hormonal therapy under the oncology board's recommendations, 98.9% received the planned radiotherapy, and 92.4% completed the full course of the prescribed treatment.

Patients who experienced disease progression were re-evaluated by the multidisciplinary oncology board, and subsequent management was guided by current oncological guidelines and drug availability. Systemic treatments most frequently included docetaxel, abiraterone, and other standard therapies for advanced PC. Subsequent therapies administered after disease progression did not affect progression-free survival (PFS) but may have influenced overall survival (OS).

Pathology

Tissue samples obtained from the primary biopsy (before any treatment) of the prostate were used to analyze lymphocytic infiltration. Histopathological and immunohistochemical analyses were performed on tissue samples obtained by needle biopsy (88.9%) and transurethral resection of the prostate (TURP) (11.1%). Multiple 3 mm sections were cut using a microtome, mounted on glass slides, and sealed with paraffin. The antibodies used in this study were as follows: CD3 [mouse monoclonal anti-human CD3, Clone 4B11 – Agilent (Dako), Cat. No. K800221-2] and CD8 [rabbit polyclonal anti-human CD8, Agilent (Dako), Cat. No. K800221-2].

Tissue samples were washed in tris-buffered saline (TBS) for 3 × 3 min, and the sections were incubated with EnVision™ FLEX+ Mouse (Linker) or EnVision™ FLEX+ Rabbit (Linker), depending on whether mouse or rabbit antibody was used, for 15 min at room temperature in a humidified chamber. The sections were washed in 0.05M TBS, pH 7.6, for 3 × 3 min and incubated with EnVision™ FLEX/horseradish peroxidase detection reagent for 20 min at room temperature in a humidified chamber. After another wash in 0.05M TBS, pH 7.6, for 3 × 3 min, the sections were incubated in 3,3′-diaminobenzidine solution for 5–10 min at room temperature, rinsed in running water, contrasted with Mayer's hematoxylin for 1 min, and washed additionally in water.

After that, the tissue sections were dehydrated through a series of ethanol solutions of increasing concentrations (70%, 96%, and 100%), immersed in xylene, and then mounted with a suitable synthetic resin (DPX or Neo-Mount) and a coverslip.

If the target antigen was present, a chromogen precipitate appeared at the site, showing brown color in contrast to the surrounding tissue, which was blue. All slides were examined under a Leica optical microscope at ×200 magnification (10 × 20) and photographed using a Leica camera, with a field of view measuring 548.98 × 412.36 μm (0.226377 mm²). The counting of lymphocytes in the field of view was performed using the commercially available software Aperio 12.0. Counting was done by manually marking each stained lymphocyte with a marker, and the software automatically counted the marked lymphocytes. T lymphocytic infiltration (including total $CD3^+$ T cells and the $CD8^+$ T-cell subset) was counted at the greatest density within TT and at IM. The area of the IM is defined as 0.5 mm within the tumor and 1 mm surrounding the TT. Necrotic regions were excluded from the evaluation.

Ethical statement

The study was approved by the Ethics Committee of the University Clinical Center of the Republic of Srpska, Banja Luka, Republic of Srpska, Bosnia and Herzegovina (No. 01-9-546-2/18, from November 2, 2018).

Statistical analysis

The Kolmogorov-Smirnov test was done to estimate deviation from the normal distribution. Since most variables were not normally distributed, nonparametric statistics (Spearman's coefficient of rank correlation – rho) were also used. All the tests were two-sided, and a $p < 0.05$ was considered statistically significant. Receiver operating characteristic (ROC) analysis was applied to assess the cut-off point for $CD3^+$ infiltration at the IM ($CD3^+$ IM value) in relation to cancer-specific death (yes/no). The same analysis was used to evaluate OS and PFS relative to $CD3^+$ IM status (low vs. high lymphocytic infiltration). The area under the curve (AUC) was calculated to estimate the discriminatory power of lymphocytic infiltration without considering the specificity and sensitivity parameters. Kaplan-Meier survival analysis was performed to estimate patient survival probabilities, considering OS, PFS, and survival time. Progression status (yes/no) was used as the endpoint, with $CD3^+$ IM status as the grouping variable. Statistical analysis was done using MedCalc version 22.0.2. (MedCalc Software, Mariakerke, Belgium) and Paleontological Statistics (PAST) version 4.17 software [8] and Statistics Kingdom software [9].

Results

An overview of the demographic, clinical, and histopathological characteristics is presented in Table 1.

In our study, patients ranged in age from 55 to 90 years, with a mean age of 69.59 ± 7.03 years. Among them, 68.9% were aged 65 or above. The pathohistological diagnosis was established from biopsy tissue samples in 88.9% of cases, and from TURP specimens in 11.1%. Patients with localized,

Table 1

Demographic, clinical, and histopathological characteristics of the patients

Parameter	Value
Age, years	
< 65	28 (31.1)
65–80	54 (60.0)
> 80	8 (8.9)
Type of biopsy	
needle biopsy	80 (88.9)
TURP	10 (11.1)
Stage of disease	
localized (T1N0M0, T2N0M0)	30 (33.3)
local advanced (T3N0M0, T4N0M0)	30 (33.3)
metastatic (TxN1M0, TxNxM1)	30 (33.3)
Initial PSA, ng/mL	
< 10	17 (18.9)
10–20	13 (14.4)
> 20	60 (66.7)
Grade group	
I	8 (8.8)
II	13 (14.4)
III	12 (13.3)
IV	19 (21.1)
V	38 (42.2)
Gleason score	
6	8 (8.8)
7	25 (27.7)
8	19 (21.1)
9	29 (32.2)
10	9 (10.0)
Perineural invasion	
yes	58 (64.4)
no	32 (35.6)
Nadir PSA, ng/mL	
< 0.5	67 (74.4)
0.5–4	12 (13.3)
> 4	11 (12.2)
Nadir testosterone, ng/mL	
> 0.5	2 (2.2)
0.5–0.2	10 (11.1)
< 0.2	78 (86.7)
Progression disease	
yes	46 (51.1)
no	44 (48.9)
PFS, months	68.94 ± 43.37 (5 to 120)
Death caused by cancer	
yes	34 (37.8)
no	56 (62.2)
OS, months	81.26 ± 39.49 (11 to 120)

TURP – transurethral resection of the prostate; PSA – prostate-specific antigen; PFS – progression-free survival; OS – overall survival.
Values are presented as numbers (percentages), except for PFS and OS, which are shown as mean ± standard deviation (range).

locally advanced, and metastatic disease were equally represented. Initially, 66.7% of patients had highly elevated PSA values > 20 ng/mL. High-grade groups (IV and V) and high GSs (8–10) were present in 63.3% of patients. Perineural invasion was observed in 64.4% of patients. ADT resulted in PSA nadir levels below 0.5 ng/mL in 74.4% of patients and testosterone nadir levels below 0.2 ng/mL in 86.7% of patients. During the ten-year follow-up period, disease progression occurred in 51.1% of patients, while death caused by carcinoma was recorded in 37.8% of patients. Demographic and cytopathological characteristics according to groups are presented in Table 2.

Figure 1 shows the difference between low (Figure 1A, 1B) and high (Figure 1C, 1D) $CD3^+$ T-cell infiltration at the IM of PC, before and after manual marking and counting of $CD3^+$ cells.

Table 2

Demographic, clinical, and histopathological characteristics according to groups

Parameter	Mean	Median	SD	Min–Max
Age, years				
A	70.47	70.5	7.45	55–90
B	69.93	69	6.69	58–80
C	68.37	67	6.99	56–84
total	69.59	68	7.03	55–90
$CD3^+$ IM*				
A	81.07	38.5	91.64	7–357
B	136.07	77.5	188.33	10–790
C	179.53	128	216.72	3–1,017
total	132.22	75	176.75	3–1,017
$CD3^+$ TT*				
A	285.9	218	308.46	19–1,190
B	244.6	148	286.31	15–1,312
C	294.87	231.5	250.21	5–1,091
total	275.12	194.5	280.35	5–1,312
$CD8^+$ IM*				
A	35.5	23	26.84	6–114
B	31.33	25	28.93	0–114
C	54.63	38	52.5	3–251
total	40.49	27	38.86	0–251
$CD8^+$ TT*				
A	105.97	76	114.79	5–539
B	84.53	52.5	117.56	0–571
C	106	62.5	103.3	0–402
total	98.83	61.5	111.25	0–571
Gleason score				
A	7.07	7	0.91	6–9
B	8.43	9	1.01	7–10
C	8.7	9	0.88	7–10
total	8.07	8	1.17	6–10
ISUP grading				
A	2.5	2	1.31	1–5
B	4.2	5	1.03	2–5
C	4.5	5	0.78	2–5
total	3.73	4	1.37	1–5
Initial PSA				
1	14.6	10.27	10.11	4.8–52
2	49.03	29.5	59.78	3.6–311
3	1,023.95	268.25	2,224.28	2.8–9,824
total	362.53	27.25	1,354.5	2.8–9,824
PFS				
A	104.93	120	28.98	27–120
B	76.77	74.5	34.39	21–120
C	25.13	18.5	19	5–73
total	60.03	60	34.17	5–120

Table 2 (continued)

Parameter	Mean	Median	SD	Min–Max
OS				
A	109.03	120	26.26	27–120
B	89.04	98.5	33.59	21–120
C	45.33	42	27.68	11–120
total	81.26	87.5	39.49	11–120

$CD3^+$ IM – number of $CD3^+$ T lymphocytes at the invasive margin (IM); $CD3^+$ TT – number of $CD3^+$ T lymphocytes in the tumor tissue (TT); $CD8^+$ IM – number of $CD8^+$ T lymphocytes at the IM; $CD8^+$ TT – number of $CD8^+$ T lymphocytes in the TT; ISUP – International Society of Urological Pathology; Group A – medium/high-risk localized disease (30 patients); Group B – locally advanced disease (30 patients); Group C – metastatic disease (30 patients); SD – standard deviation; Min – minimum; Max – maximum.
For other abbreviations, see Table 1.
***Note*: *$CD3^+$ and $CD8^+$ values represent the number of T lymphocytes *per* field of view (0.226377 mm^2).**

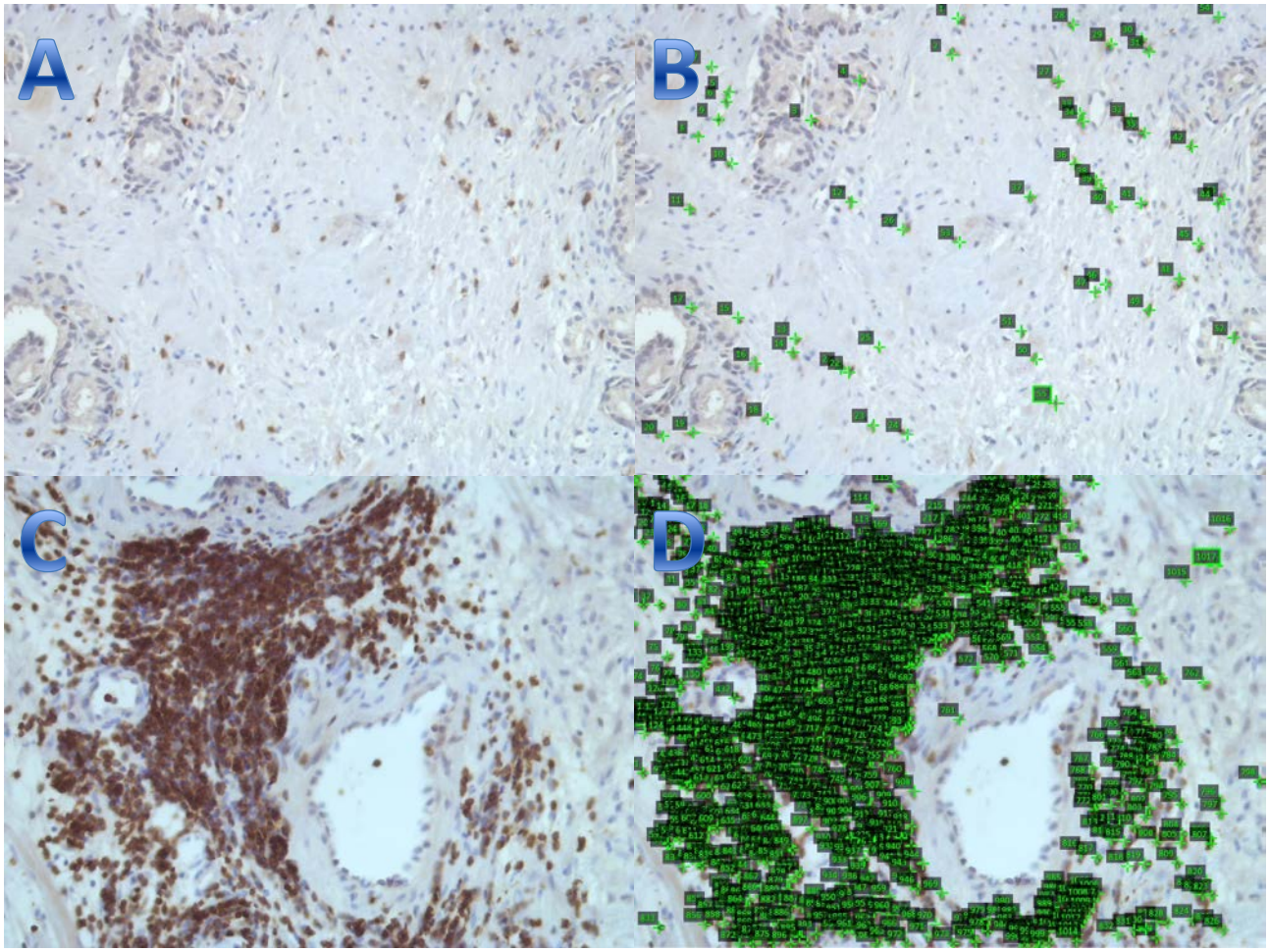

Fig. 1 – Immunohistochemical detection and analysis of $CD3^+$ T lymphocytes infiltration at the invasive margin in prostate cancer tissue. Low lymphocytic infiltration before (A) and after (B) manual marking and quantification of $CD3^+$ T lymphocytes. High $CD3^+$ T lymphocytes infiltration before (C) and after (D) manual marking and quantification. $CD3^+$ T cells were detected using 3,3′-diaminobenzidine immunostaining (brown), with hematoxylin counterstaining (blue). Images, captured at ×200 magnification, were analyzed using Aperio 12.0 software (field of view size: 0.226377 mm^2).

Differences in the number of $CD3^+$ T lymphocytes at the IM were analyzed among three groups (A, B, and C) of PC patients (Figure 2). Significant differences in lymphocytic infiltration were observed between different disease stages (groups), particularly when comparing localized/locally advanced disease with metastatic PC.

The results of the correlation coefficient among observed parameters showed statistically significant correlations between $CD3^+$ IM and PFS, as well as $CD3^+$ IM and OS (Table 3), indicating that higher $CD3^+$ IM values were associated with shorter PFS and OS. There is also a correlation between the number of $CD3^+$ IM T lymphocytes and disease stage, perineural infiltration, initial PSA, and nadir PSA. In contrast, no correlation was found between the number of $CD3^+$ IM T lymphocytes and GS or ISUP grade. No statistically significant correlations with PFS or OS were found for $CD3^+$ infiltration in TT, nor for $CD8^+$ T lymphocyte infiltration at either the TT or IM (Table 3).

The ROC analysis was performed to evaluate whether the $CD3^+$ IM value has discriminatory potential with respect to cancer-specific mortality. The result of the ROC analysis indicated statistically significant differentiation reliability [AUC = 0.681, standard error (SE) = 0.0577, 95% confidence interval (CI): 0.575 to 0.776, $p = 0.0017$], where a cut-off value > 73 was observed for maximum sensitivity of 72.73 and specificity of 63.17 (Figure 3).

This result suggests that $CD3^+$ IM values above 73 are predictive of cancer-related death with relatively high specificity and sensitivity. We defined two groups based on the cut-off value of $CD3^+$ IM: low lymphocytic infiltration and high lymphocytic infiltration. The ROC analysis was performed using OS and PFS as criteria.

When OS was used as the criterion, ROC analyses showed statistically significant discriminatory power (AUC = 0.634, SE = 0.0563, 95% CI: 0.526 to 0.733, $p = 0.0173$). A cut-off value ≤ 54 was observed for maximum sensitivity of 46.67 and specificity of 80.00 (Figure 4). In case of PFS, the same analyses also showed statistically significant discriminatory power (AUC = 0.657, SE = 0.0574, 95% CI: 0.550 to 0.754, $p = 0.0062$). The cut-off value ≤ 34 was identified for a maximum sensitivity of 48.89 and a specificity of 84.44 (Figure 5).

The results of the Kaplan-Meier analysis showed that when the survival time was defined as PFS, and progression status (yes/no) was used as the endpoint, $CD3^+$ IM status (low vs. high lymphocytic infiltration) was a significant indicator of PFS. The difference between the groups was statistically significant (log-rank test: $\chi^2 = 11.75$, $p = 0.0006$) (Figure 6). A total of 15 patients (8 in the low lymphocyte infiltration group and 7 in the high lymphocyte infiltration group) who died of other causes without prior disease progression were excluded from the analysis. The average PFS for patients with low lymphocytic infiltration was 81.16 months, and for the group with high lymphocytic infiltration was 56.73 months. In patients with low lymphocytic infiltration, 120 months without progression was reached by 64.4% of patients. During the same period, among the patients with high lymphocytic infiltration, 33.3% remained progression-free for 120 months.

The same result was obtained when OS was considered as survival time (log-rank test – $\chi 2 = 13.429588$, $p = 0.000024787$) (Figure 7). Patients who died due to another cause (15 patients) were excluded from the analysis. The average OS for patients with low lymphocytic

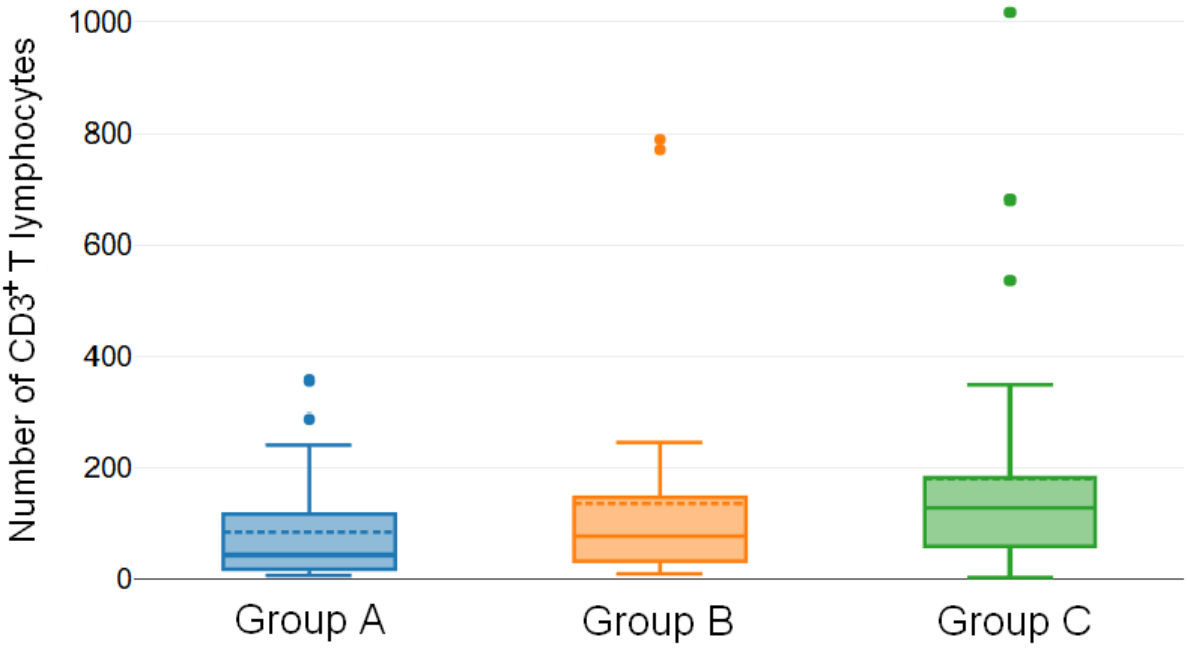

Fig. 2 – Differences in the number of $CD3^+$ T lymphocytes at the invasive margin according to disease stage. Group A – medium/high-risk localized disease; Group B – locally advanced disease; Group C – metastatic disease.

Table 3

Correlation coefficients between T lymphocyte infiltration and patient clinical/pathological data

Parameters	$CD3^+$ IM	$CD3^+$ TT	$CD8^+$ IM	$CD8^+$ TT
PFS	ρ = -0.2216 95% CI: -0.4145 to -0.0097 *p* = 0.03579*	ρ = -0.1049 95% CI: -0.3086 to 0.1079 *p* = 0.325	ρ = - 0.115 95% CI: -0.31791 to 0.09792 *p* = 0.2804	ρ = -0.01874 95% CI: -0.2278 to 0.1919 *p* = 0.8608
OS	ρ = -0.2146 95% CI: -0.4083 to-0.002522 *p* = 0.04221*	ρ = -0.1044 95% CI: -0.3081 to 0.1084 *p* = 0.3274	ρ = -0.1602 95% CI: -0.3592 to 0.0528 *p* = 0.1315	ρ = -0.02125 95% CI: -0.2301 to 0.1895 *p* = 0.8424
Stage of disease	ρ = 0.3036 95% CI: 0.09528 to 0.4864 *p* = 0.003627*	ρ = 0.06312 95% CI: -0.149 to 0.2696 *p* = 0.5933	ρ = 0.1787 95% CI: -0.03412 to 0.376 *p* = 0.09198	ρ = 0.01493 95% CI: -0.1956 to 0.2241 *p* = 0.8889
Age	ρ = -0.033 95% CI: -0.2414 to 0.1781 *p* = 0.7569	ρ = -0.1093 95% CI: -0.3126 to 0.1036 *p* = 0.305	ρ = -0.1026 95% CI: -0.3064 to 0.1102 *p* = 0.3359	ρ = -0.05281 95% CI: -0.26 to 0.159 *p* = 0.621
Gleason score	ρ = 0.1197 95% CI: -0.09326 to 0.3222 *p* = 0.2611	ρ = -0.003977 95% CI: -0.2137 to 0.2061 *p* = 0.9703	ρ = -0.06511 95% CI: -0.2715 to 0.147 *p* = 0.5421	ρ = -0.01861 95% CI: -0.2276 to 0.1921 *p* = 0.8618
ISUP grading	ρ = 0.1302 95% CI: -0.08283 to 0.3318 *p* = 0.2213	ρ = 0.01553 95% CI: -0.195 to 0.2247 *p* = 0.8845	ρ = -0.03539 95% CI: -0.2435 to 0.1759 *p* = 0.7406	ρ = -0.01146 95% CI: -0.2208 to 0.1989 *p* = 0.9147
Perineural infiltration	ρ = 0.2994 95% CI: 0,09079 to 0.4828 *p* = 0.004152*	ρ = 0.2399 95% CI: 0.02856 to 0.4307 *p* = 0.02276*	ρ = 0.09921 95% CI: -0.1136 to 0.3033 *p* = 0.3522	ρ = 0.1251 95% CI: -0.08791to 0.3272 *p* = 0.2401
Initial PSA	ρ = 0.2517 95% CI: 0.04082 to 0.4412 *p* = 0.01669*	ρ = 0.08614 95% CI: -0.1264 to 0.2911 *p* = 0.4195	ρ = 0.1267 95% CI: -0.0863 to 0.3286 *p* = 0.234	ρ = 0.01447 95% CI: -0.196 to 0.2237 *p* = 0.8923
Nadir PSA	ρ = 0.2825 95% CI: 0.07299 to 0.4681 *p* = 0.006982*	ρ = 0.1188 95% CI: -0.09415 to 0.3214 *p* = 0.2647	ρ = 0.1595 95% CI: -0.05347 to 0.3586 *p* = 0.1332	ρ = 0.02123 95% CI: -0.1895 to 0.2301 *p* = 0.8425
Nadir testosterone	ρ = 0.0616 95% CI: -0.1504 to 0.2682 *p* = 0.5641	ρ = 0.02959; 95% CI: -0.1815 to 0.238 *p* = 0.7819	ρ = -0.07021 95% CI: -0.2072 to 0.2072 *p* = 0.5108	ρ = -0.03583 95% CI: -0.244 to 0.1754 *p* = 0.7374

CI – confidence interval.
For other abbreviations, see Tables 1 and 2.
Statistical test, Spearman's coefficient of rank correlation (rho), was used.
***Note*: *statistically significant value.**

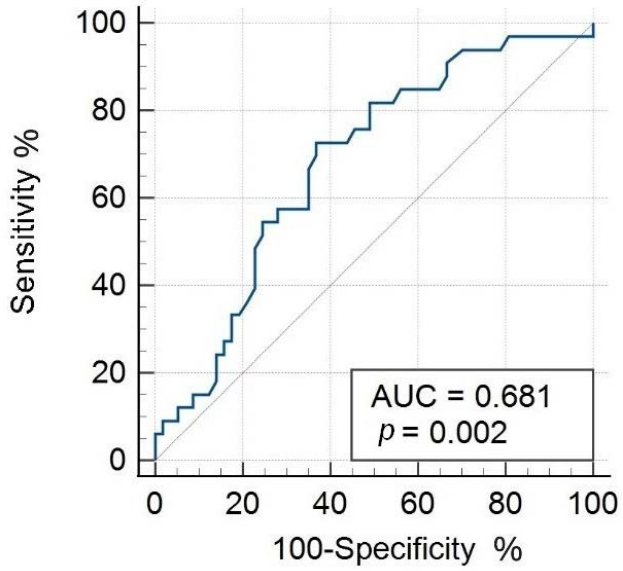

Fig. 3 – Receiver operating characteristic curve for CD3⁺ T infiltration at the invasive margin of prostate cancer tissue. AUC – area under the curve.

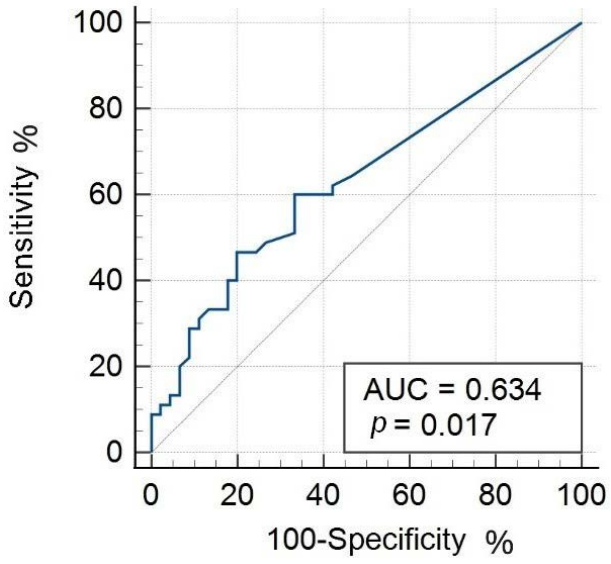

Fig. 4 – Receiver operating characteristic curve for overall survival. AUC – area under the curve.

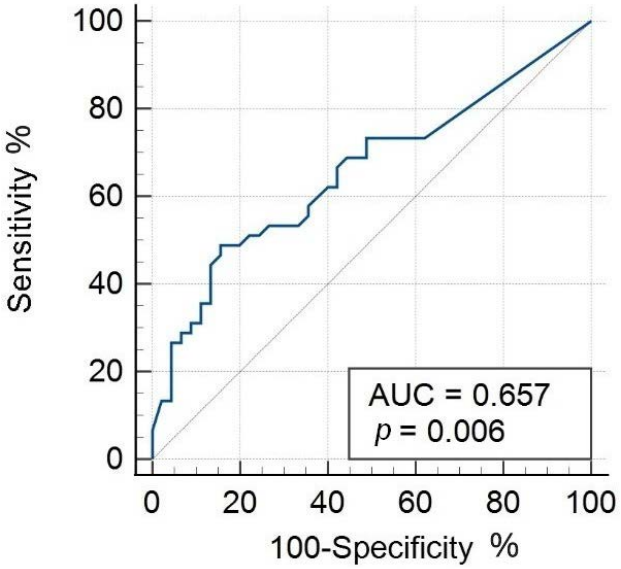

Fig. 5 – Receiver operating characteristic curve for progression-free survival. AUC – area under the curve.

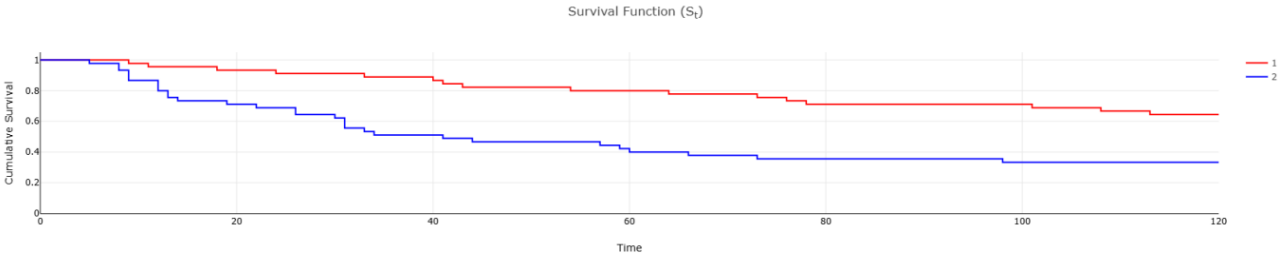

Fig. 6 – Kaplan-Meier analysis of progression-free survival based on CD3⁺ T-cell infiltration at the invasive margin (IM).
***Note*: Patients were grouped according to CD3⁺ IM status (low vs. high), with progression status (yes/no) used as the event endpoint. The red and blue lines indicate groups with low and high lymphocyte infiltration, respectively.**

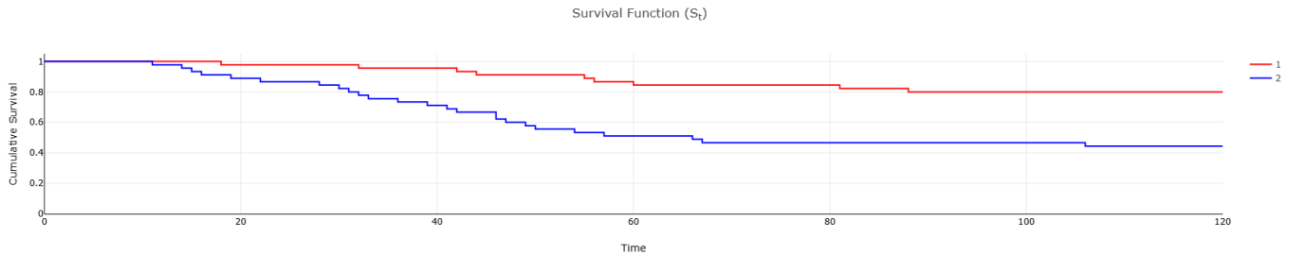

Fig. 7 – Kaplan-Meier analysis of overall survival based on CD3⁺ T-cell infiltration at the invasive margin (IM). ***Note*****: Patients were grouped according to CD3⁺ IM status (low vs. high), with progression status (yes/no) used as the event endpoint. The red and blue lines indicate groups with low and high lymphocyte infiltration, respectively.**

infiltration was 91.36 months, and 71.16 months for patients with high lymphocytic infiltration. In a group of patients who had low lymphocytic infiltration, 120 months of OS were reached by 80% of patients. During the same period, among patients with high lymphocytic infiltration, 44% achieved a total survival of 120 months.

Discussion

For a long time, PC was considered an immunologically "cold" tumor. In recent years, however, TILs, particularly T lymphocytes, have become the focus of intense investigation. In our study of 90 patients with localized, locally advanced, and metastatic PC, where all lymphocytes were counted in the areas of highest density (within TT and at IM), we found that lymphocyte infiltration was not abundant. Based on our findings, we agree that PC is not an immune cell-enriched tumor, showing only limited TIL infiltration, as supported by the literature [10].

Regarding CD8⁺ T cells, our analysis did not find a significant association between CD8⁺ T cell infiltration in TT or at IM and clinical outcomes such as PFS or OS. This aligns with the results reported by Davidsson et al. [11], who found no correlation between CD4⁺ and CD8⁺ T cell infiltration and survival in a case-control study. Conversely, our findings contradict those of Yang et al. [12], who reported CD8⁺ T infiltration as a favorable prognostic factor for OS. Such discrepancies may be attributed to differences in patient populations, sample types, treatment regimens, or TIL quantification methods.

However, the situation differs in relation to CD3⁺ T lymphocytes. The results of correlation analyses among observed parameters showed statistically significant associations between CD3⁺ IM and PFS, as well as CD3⁺ IM and OS, in such a way that higher values of CD3⁺ IM corresponded to lower values of PFS and OS. In our research, we proved that CD3⁺ T lymphocytic infiltration on IM (peritumoral tissue) makes a statistically significant difference in PFS and OS, which correlates with the findings of Andersen et al. [2] who indicated that different immune cell infiltration patterns in stroma (peritumoral) and epithelium (intratumorally) have a complex biological role for the development and/or progression of PC. Similar results were reported by Kärjä et al. [13], who found that low TIL expression was associated with local disease and indicated a favorable clinical behavior. Richardsen et al. [14] confirm that a high density of CD3⁺ T lymphocytes in tumor cell areas and tumor stromal areas of PC correlated with metastatic disease. In more than 3,000 patients, Flammiger et al. [15] investigated the prognostic effects of TILs in PC. They found that patients with very low and very high densities of intratumoral CD3⁺ T cells had a significantly shorter recurrence period (measured as an increase in PSA level), unlike patients with intermediate numbers of T cells. However, they did not investigate the influence of different subtypes of T lymphocytes on the clinical outcome. Long-term study Molina et al. [16] showed that increased infiltration of CD3⁺ T lymphocytes correlated with a worse prognosis of PC, but CD8⁺ T lymphocytes had no impact on long-term OS. In our study, we found that infiltration of CD3⁺ T lymphocytes at IM is an independent predictor of shorter PFS and OS, and such patients require a more intensive therapeutic approach. For CD3⁺ T cells in TT and CD8⁺ T cells in TT and at IM, we did not find a statistically significant correlation with PFS and OS. In our study, no correlation was found between lymphocytic infiltration and patient age, in contrast to the findings reported by Soliman et al. [3]. However, a correlation was confirmed between CD3⁺ T lymphocytes at IM and both the initial PSA and nadir PSA values, as well as with perineural invasion. These findings suggest that CD3⁺ T lymphocytes at IM may play a role not only in the immune response but also in the biological behavior and aggressiveness of the tumor. The absence of correlation with patient age indicates that lymphocytic infiltration is more closely associated with tumor-related factors than with host age-related immune variability.

Limitations

This study has several limitations. The relatively small sample size (n = 90) and single-center design may limit the generalizability of results. Tissue samples for immunohistochemical analysis were obtained *via* needle biopsy and TURP, unlike many other studies that used radical prostatectomy specimens, which may better represent the entire tumor microenvironment. This difference in sampling could affect the assessment of lymphocytic infiltration. Clinical TNM staging was determined based on physical examination and imaging,

without pathological confirmation, which may affect staging accuracy. Only $CD3^+$ and $CD8^+$ markers were analyzed, without broader immune profiling. Furthermore, infiltration was evaluated only at baseline, with no longitudinal follow-up of the immune response during treatment. All patients were initially treated with hormonal suppression and radiotherapy, as these were the only available treatment options 11 to 13 years ago when their therapy began. Today, treatment strategies would likely differ, which may limit the generalizability of our findings to current clinical practice. Lastly, while the impact on PFS was analyzed, post-progression therapies may have influenced OS outcomes.

Conclusion

In our research, we demonstrated that peritumoral infiltration of $CD3^+$ lymphocytes is an independent predictor of shorter PFS and OS in PC patients. This infiltration also shows significant correlation with both prognostic and predictive factors, including initial PSA levels, perineural invasion, and the achievement of maximal disease regression, as indicated by nadir PSA.

Conflict of interest

The authors declare no conflict of interest.

R E F E R E N C E S

1. *Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I,* et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2024; 74(3): 229–63.
2. *Andersen LB, Nørgaard M, Rasmussen M, Fredsøe J, Borre M, Ulhøi BP,* et al. Immune cell analyses of the tumor microenvironment in prostate cancer highlight infiltrating regulatory T cells and macrophages as adverse prognostic factors. J Pathol 2021; 255(2): 155–65.
3. *Soliman SAM, Yassen NN, Abdelaal SE, Shabana ME.* Expression of CD3+ and CD8+ tumor-infiltrating lymphocytes in prostatic carcinoma: immunohistochemical study and image analysis. Egypt J Pathol 2024; 44(2): 207–14.
4. *Ness N, Andersen S, Valkov A, Nordby Y, Donnem T, Al-Saad S,* et al. Infiltration of CD8+ lymphocytes is an independent prognostic factor of biochemical failure-free survival in prostate cancer. Prostate 2014; 74(14): 1452–61.
5. *Zafar MM, Rauf Z, Sohail A, Khan AR, Obaidullah M, Khan SH,* et al. Detection of tumour infiltrating lymphocytes in CD3 and CD8 stained histopathological images using a two-phase deep CNN. Photodiagnosis Photodyn Ther 2022; 37: 102676.
6. *Esmail RSEN, Kamal A, Shabana M.* Immunoscore As A Predictor Of Disease Recurrences And Patientsâ€™ Survival in Colon Cancer: A Clinicopathologic Study. Open Access Maced J Med Sci 2020; 8(E): 143–9.
7. *Lam TBL, MacLennan S, Willemse PM, Mason MD, Plass K, Shepherd R,* et al. EAU-EANM-ESTRO-ESUR-SIOG Prostate Cancer Guideline Panel Consensus Statements for Deferred Treatment with Curative Intent for Localised Prostate Cancer from an International Collaborative Study (DETECTIVE Study). Eur Urol 2019; 76(6): 790–813.
8. *Hammer Ø, Harper DAT, Ryan PD.* PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica 2001; 4(1): 1–9.
9. *Statistics Kingdom.* Correlation Coefficient Calculator [Internet]. Melbourne: Statistics Kingdom [cited 2025 Jun 23; accessed 2025 Aug 28]. Available from: https://www.statskingdom.com/correlation-calculator.html
10. *Vilaça M, Correia Pinto J, Magalhães H, Reis F, Mesquita A.* Tumor-Infiltrating Lymphocytes in Localized Prostate Cancer: Do They Play an Important Role? Cureus 2023; 15(1): e34007.
11. *Davidsson S, Ohlson AL, Andersson SO, Fall K, Meisner A, Fiorentino M,* et al. CD4 helper T cells, CD8 cytotoxic T cells, and FOXP3(+) regulatory T cells with respect to lethal prostate cancer. Mod Pathol 2013; 26(3): 448–55.
12. *Yang Y, Attwood K, Bshara W, Mohler JL, Guru K, Xu B,* et al. High intratumoral CD8+ T-cell infiltration is associated with improved survival in prostate cancer patients undergoing radical prostatectomy. Prostate 2021; 81(1): 20–8.
13. *Kärjä V, Aaltomaa S, Lipponen P, Isotalo T, Talja M, Mokka R.* Tumour-infiltrating lymphocytes: A prognostic factor of PSA-free survival in patients with local prostate carcinoma treated by radical prostatectomy. Anticancer Res 2005; 25(6C): 4435–8.
14. *Richardsen E, Uglehus RD, Due J, Busch C, Busund LT.* The prognostic impact of M-CSF, CSF-1 receptor, CD68 and CD3 in prostatic carcinoma. Histopathology 2008; 53(1): 30–8.
15. *Flammiger A, Bayer F, Cirugeda-Kühnert A, Huland H, Tennstedt P, Simon R,* et al. Intratumoral T but not B lymphocytes are related to clinical outcome in prostate cancer. APMIS 2012; 120(11): 901–8.
16. *Molina OE, LaRue H, Simonyan D, Hovington H, Vittrant B, Têtu B,* et al. Regulatory and memory T lymphocytes infiltrating prostate tumors predict long term clinical outcomes. Front Immunol 2024; 15: 1372837.

Received on March 15, 2025
Revised on July 15, 2025
Revised on August 19, 2025
Accepted on September 3, 2025
Online First October 2025