

A Multi-Biomarker Signature of Immune Activation and Metabolic Shift Predicts Treatment Success in Localized Prostate Cancer

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Abstract

Background: Prostate cancer's response to therapy varies significantly, and traditional markers like PSA offer limited prognostic insight. A thorough understanding of systemic immune and metabolic changes post-treatment is essential for developing better predictive tools. **Objective:** To characterize the profiles of circulating immune and metabolic biomarkers prior to and following definitive therapy in patients with localized prostate cancer, and to assess their efficacy in distinguishing biochemical responders from non-responders. **Materials and Methods:** This prospective study involved 90 patients with prostate adenocarcinoma treated at the Oncology Teaching Hospital / Baghdad Teaching Hospital, between February 2024 and February 2025. Patients underwent radical prostatectomy or radiotherapy, with or without androgen deprivation therapy. Blood samples were collected at baseline and three months post-treatment to measure six biomarkers (GFRAL, adenosine (AD), interleukin-39 (IL-39), TRAIL, lipopolysaccharide (LPS), and SDF1) using ELISA. Treatment response was defined as PSA below 6.5 ng/mL. Data were analyzed using nonparametric tests, machine learning, ROC analysis, and multivariate regression, with stratification by treatment type to evaluate intervention-specific changes in biomarkers. **Results:** Following treatment, responders exhibited a distinct molecular profile characterized by significantly increased levels of SDF1 and IL-39, along with decreased concentrations of AD and LPS. SDF1 was identified as the most reliable individual predictor, with an area under the curve (AUC) of 0.89. A multivariable model including SDF1, AD, and IL-39 demonstrated improved predictive performance, achieving an AUC of 0.92, with a sensitivity of 89.1% and specificity of 85.7%. Multivariate analysis confirmed that SDF1 and AD are independent predictors of response, after adjusting for age, Gleason score, baseline PSA, and treatment modality. **Conclusion:** Effective prostate cancer treatment prompts a systemic immunometabolic transformation towards a reparative and immune-active state. A multi-biomarker panel, notably including SDF1, AD, and IL-39, emerges as a promising discovery-phase signature that, following independent external validation, may provide a non-invasive method for monitoring therapeutic response, but further validation is needed before clinical use.

Keywords: Prostate Cancer, Biomarkers, SDF1 (CXCL12), Adenosine, Immunometabolic Profiling, Treatment Response

Asian Pac J Cancer Biol, **11** (4), 1021-1035

Submission Date: 07/23/2026

Acceptance Date: 09/03/2026

1. Introduction

The management of prostate cancer is hampered by the remarkable heterogeneity in response to definitive local therapies such as radical prostatectomy and radiotherapy [1]. Although the goal of treatment is curative, outcomes can vary widely since many individuals develop

biochemical recurrence [2], emphasizing the need for better prognostic stratification. Typical limitations of prostate-specific antigen as an individual marker are clear with low specificity and lack of reflection upon disease aggressiveness [3, 4]. More importantly, PSA

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is a late surrogate of tumor burden that says nothing about the systemic host response and especially the immunometabolic reprogramming now known to be essential for therapeutic effectiveness [5, 6]. Dependence on a biomarker with minimal prognostic value complicates clinical decision-making, impedes early identification of treatment failure and prolongs delay in salvage therapy initiation, highlighting the necessity for dependable and plausible predictive resources demonstrating better correlation to treatment response [7].

This recognition of the weaknesses associated with PSA has heralded a change in thinking when it comes to treating concepts of cancer. Now, success is measured not only in (removing) tumors but also inducing systemic responses that reset the balance of body systems. This emerging view underscores the coordinated immune and metabolic rewiring supporting durable responses, a recognition that transcends tumor biology to how we should treat cancer as a disease holistically [8]. These systemic changes play out in the circulatory system, where concentrations of certain immune signaling molecules and metabolic intermediates become dynamic, real-time measures of a host's response [9]. The systemic immunometabolic reprogramming has given rise to the conclusion that circulating markers represent a search for molecules with potential connection with tissue recovery and immune cells recruitment [10, 11], such as SDF-1 (CXCL12), or adenosine acting as tumors' immunosuppressant.

Herein, we develop an immunometabolome-guided biomarker with a focus on six biological domains relevant to prostate cancer treatment response. This panel consists of IL-39, a cytokine belonging to the IL-12 family, and its effects on inflammation and tumor immunity are of great interest [12]. Lipopolysaccharide (LPS), a component of the cell wall of Gram-negative bacteria, is a marker for microbial translocation and systemic inflammation, both correlating with progression to prostate cancer recurrence and castrate resistance [13]. The known receptor of GDF15, GDNF Family Receptor Alpha-Like (GFRAL), was added to assess pathways involved in metabolic stress and cachexia that impact treatment outcome [14]. TRAIL (TNF-related apoptosis-inducing ligand) was assessed to ensure extrinsic apoptotic pathways relevant to radiotherapy and systemic treatments were intact [15]. These biomarkers combined provide a global assessment of host-tumor interactions, with profiling immune activation (IL-39), inflammatory load (LPS), metabolic stress (GFRAL) and apoptosis (TRAIL). Transforming these biomarkers into a clinical tool needs solid methods and mechanistic understanding to enhance tailored and predictive treatment options.

Studying circulating biomarkers offers a valuable, non-invasive approach to advancing this paradigm. Unlike static, tumor-centric markers like PSA, circulating biomarkers enhance understanding of host-tumor interactions through proteomic and metabolic blood profiling. These molecules reflect systemic responses and tumor microenvironment dynamics, offering a comprehensive, mechanistic view of therapeutic efficacy

beyond tumor size, including immune activation and suppression [9]. The robustness and scalability of modern immunoassay platforms support the idea of cancer being a systemic disease. Techniques such as ELISA enable precise, sensitive measurement of circulating biomarkers, facilitating clinical applications. Their widespread use supports multi-biomarker profiling, helping shift cancer diagnostics from research to everyday clinical use [10]. Using a panel of biomarkers measured through ELISA, clinicians can establish a dynamic, composite signature of the host response. This approach shifts from passive observation to an active, biologically informed strategy aimed at personalizing prostate cancer management and enabling early detection of therapeutic resistance.

The full potential of circulating biomarker panels depends on analytical methods capable of addressing their inherent complexity. Effective synthesis of raw data into clinically interpretable and personalized predictions is crucial. Traditional univariate statistical methods often fail to capture complex, non-linear interactions among biomarkers and clinical variables. Advanced computational techniques, such as machine learning methods including Random Forests and support vector machines, can identify subtle patterns, improving risk assessment and predicting outcomes [16, 17]. Furthermore, techniques such as principal component analysis (PCA) can reduce data complexity, highlighting the main patterns of biological variation within a group and pinpointing the most useful marker combinations. Integrating multi-biomarker panels with advanced analytical techniques is a crucial step toward truly personalized medicine, enabling data-driven, adaptable models that can improve prognosis and customize treatments in prostate cancer care.

Despite growing interest in systemic biomarkers for cancer prognosis, a critical evidence gap remains in localized prostate cancer: most studies depend on single-analyte measurements without incorporating longitudinal immune-metabolic profiles to evaluate early post-treatment response. The majority of current studies emphasize single-analyte measurements or lack systematic integration of longitudinal immune and metabolic profiles to evaluate early post-treatment responses. Consequently, there is a notable deficiency in comprehensive research that simultaneously monitors circulating signatures across multiple dimensions over time to differentiate responders from non-responders shortly after definitive therapy. This absence of integrated, longitudinal data impedes the identification of predictive signatures that reflect the dynamic host response, which is essential for timely clinical decision-making.

We hypothesize that a multi-biomarker panel reflecting coordinated systemic immunometabolic changes can differentiate biochemical responders from non-responders shortly after definitive therapy. To test this hypothesis, we conducted a prospective study to fill this gap by evaluating six circulating biomarkers (GFRAL, adenosine, IL-39, TRAIL, LPS, and SDF1) at baseline and three months post-treatment in patients undergoing radical prostatectomy or radiotherapy. Acknowledging the biological heterogeneity inherent

across treatment modalities, our analytical approach was designed to stratify biomarker responses by intervention type and incorporate modality adjustments into multivariate predictive models, ensuring that any identified signatures reflect host response biology rather than intervention-specific effects.

2. Materials and Methods

2.1. Study Design and Setting

This prospective, single-center, paired-sample biomarker study was conducted at the Oncology Teaching Hospital and Baghdad Teaching Hospital (Medical City) in Baghdad between February 2024 and February 2025. The research aimed to examine circulating immune and metabolic biomarkers before and after initial treatment in patients diagnosed with localized or locally advanced prostate cancer.

2.2. Study Population

The study involved ninety adult male patients diagnosed with prostate adenocarcinoma confirmed via histological examination. All patients were scheduled to receive definitive treatment, either through radical prostatectomy or radiotherapy, with some also undergoing androgen deprivation therapy.

a. Inclusion Criteria

The research involved ninety adult male patients with histologically confirmed prostate adenocarcinoma, all of whom were scheduled to receive definitive treatment (either radical prostatectomy or radiotherapy), potentially combined with androgen deprivation therapy.

b. Exclusion Criteria

Patients with metastatic disease, a history of previous prostate cancer treatment, active infections, autoimmune disorders, or concurrent malignancies were excluded from the study. Furthermore, the use of immunomodulatory or anti-inflammatory agents within four weeks before the study was deemed an important exclusion criterion.

2.3. Data Collection, Clinical Variables, and Biomarker Measurement:

2.3.1. Data Collection Schedule

Data and biospecimen collection occurred at two predetermined critical junctures: baseline (T0), within 2 weeks before therapeutic intervention, and follow-up (T1), at 3 months post-therapy. At each time point, both clinical data and venous blood samples were systematically collected from all participants in accordance with standardized operating procedures.

2.3.2. Clinical and Demographic Variables

The demographic and clinicopathological variables were obtained from electronic medical records and confirmed through direct patient interviews. These included age (expressed as mean $\pm$ standard deviation), histologically verified Gleason score (classified as ≤ 7 or

≥ 8 based on the 2019 International Society of Urological Pathology consensus guidelines [18], clinical TNM stage (T2- Organ-Confining Disease vs. T3- Locally Advanced Disease), and the primary treatment modality, which comprised either radical prostatectomy (RP) or radiotherapy with or without androgen deprivation therapy (RT $\pm$ ADT).

Prostate-specific antigen (PSA) levels were measured at both T0 and T1 using a standardized chemiluminescent microparticle immunoassay (Abbott Architect i2000SR, Abbott Park, IL, USA) performed in the institutional clinical laboratory [19]. All procedures adhered strictly to the manufacturer's guidelines and incorporated appropriate quality control measures.

2.3.3. Treatment Response Classification

Biochemical response was assessed three months after therapy (T1). Patients were classified as responders if their post-treatment PSA was below 6.5 ng/mL and as non-responders if their PSA was 6.5 ng/mL or higher. This threshold was determined using a data-informed approach based on the cohort's post-treatment PSA distribution. Specifically, 6.5 ng/mL corresponds to the 75th percentile of PSA levels measured 3 months after definitive therapy. This cutoff was selected a priori to balance sensitivity and specificity in the early assessment of biochemical response [20].

This distribution-based method serves two primary purposes: first, to identify the upper quartile of patients with the least favorable early PSA clearance, representing an 'at-risk' population for residual disease; and second, to create statistically balanced groups for hypothesis generation, allowing us to test whether systemic immunometabolic profiles differ between patients with adequate versus inadequate early biochemical responses.

The scientific justification for employing a three-month evaluation period is well-supported. Recent prospective research has established that PSA kinetics observed as early as three months after treatment can serve as reliable surrogate markers for predicting long-term oncological outcomes. A recent study published in *The Prostate* confirmed that both the absolute PSA level and PSA response rate at three months are significantly associated with progression-free and overall survival [21]. Similarly, large real-world analyses have shown that early PSA decline by three months predicts subsequent radiologic outcomes at 24 months [22].

While traditional endpoints such as biochemical recurrence-free survival and the Phoenix criteria (PSA nadir + 2 ng/mL) remain the gold standard for long-term follow-up, these measures are not suitable for the early (three-month) assessment period used in this study. Specifically, the Phoenix criteria were originally devised to define treatment failure following the nadir, typically 12 to 24 months post-radiotherapy, and are therefore inapplicable at the three-month mark [23].

Therefore, the 6.5 ng/mL threshold serves as a practical early-response marker that facilitates prompt identification of patients with insufficient PSA decline who may require more vigilant monitoring or earlier salvage treatment.

To confirm clinical significance, all biochemical classifications were validated through supplementary assessments, including digital rectal examination (DRE) and, when deemed necessary, multiparametric magnetic resonance imaging (MRI), to assess both localized and systemic disease status.

2.3.4. Blood Sample Processing and Biomarker Quantification

a. Sample Collection and Processing

Venous blood samples were obtained from all 90 patients and collected into sterile EDTA-containing tubes (BD Vacutainer, Franklin Lakes, NJ, USA) at both T0 and T1. Plasma was promptly separated by centrifugation at 3,000 rpm for 15 minutes at 4°C, then aliquoted into sterile cryovials and stored at -80°C to ensure consistency across batch analyses, thereby reducing inter-assay variability. All samples underwent only a single freeze-thaw cycle before analysis.

b. Biomarker Panel Selection Rationale

The six-biomarker panel was carefully chosen within a hypothesis-driven framework that targets six key biological domains involved in prostate cancer treatment response: [1] tissue repair and immune recruitment (SDF1); [2] immunosuppressive metabolism (adenosine); [3] inflammatory regulation (IL-39); [4] systemic inflammatory burden (LPS); [5] metabolic stress signaling (GFRAL); and [6] apoptotic effector function (TRAIL). This comprehensive approach aims to capture systemic immunometabolic reprogramming following definitive therapy, shifting focus from static, tumor-centered markers to dynamic host-response biology.

c. ELISA Procedures and Assay Specifications

All six circulating biomarkers were quantified using commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturers' protocols, with each sample analyzed in duplicate. Laboratory personnel conducting the assays were blinded to the patients' clinical outcomes and response classifications. Absorbance was measured at 450 nm using a BioTek Synergy HTX microplate reader (Winooski, VT, USA), and concentrations were derived from standard curves generated via four-parameter logistic regression. Detailed assay performance characteristics are provided below.

SDF1 (stromal cell-derived factor 1 alpha) was evaluated for its involvement in tissue repair and immune cell recruitment. Quantification was performed using the human SDF1 α ELISA kit (Abcam, Cambridge, UK; catalog number ab100637), with a detection range of 0.156–10 ng/mL. The intra-assay coefficient of variation (CV) was less than 7%, and the inter-assay CV was also determined [24]. Adenosine (AD) was evaluated as a key immunosuppressive metabolite within the tumor microenvironment. Quantification was performed using a human adenosine ELISA kit (Cusabio, Houston, TX, USA; catalog number CSB-E13075h), with a detection range of

15.6–1000 ng/mL. The assay demonstrated an intra-assay coefficient of variation (CV) of less than 8% and an inter-assay CV of less than 10% [25]. Interleukin-39 (IL-39) was evaluated as a regulator of inflammatory responses and immune polarization. Quantification was performed using a human IL-39 ELISA kit (MyBioSource, San Diego, CA, USA; catalog number MBS3804689), with a detection range of 0.3–90 pg/mL and intra- and inter-assay coefficients of variation of 6% and 9%, respectively [26]. Lipopolysaccharide (LPS) was assessed as a surrogate marker for microbial translocation and systemic inflammatory burden. Quantification was performed using a human LPS ELISA kit (Hycult Biotech, Uden, The Netherlands; catalog number HK315) with a detection range of 0.32–20 ng/mL. The assay demonstrated intra-assay and inter-assay coefficients of variation (CVs) of less than 9% [27]. GFRAL (GDNF family receptor alpha-like) was examined for its role in metabolic stress signaling and as the known receptor for GDF15. The protein levels were quantified using a human GFRAL ELISA kit (R&D Systems, Minneapolis, MN, USA; catalog number DY10863), with a detection range of 0.312–20 ng/mL and intra-assay and inter-assay coefficients of variation of less than 8% and 10%, respectively [28]. TRAIL (TNF-related apoptosis-inducing ligand) was assessed to evaluate the integrity of the extrinsic apoptotic pathways. Quantification was performed using a human TRAIL ELISA kit (Invitrogen, Carlsbad, CA, USA; cat. BMS2004), with a detection range of 0.156–10 ng/mL. The intra-assay coefficient of variation was less than 6%, and the inter-assay coefficient of variation was less than 8%. [29].

2.3.5. Treatment Protocol and Response Assessment

All participants in the study received treatment with curative intent for histologically confirmed prostate adenocarcinoma, following established institutional guidelines [30]. Treatment options encompassed either radical prostatectomy (RP) or definitive radiotherapy (RT), with or without the addition of androgen deprivation therapy (ADT). The selection was informed by multidisciplinary tumor board recommendations and tailored to individual clinical factors, including Gleason score, staging, and comorbid conditions. Radiotherapy was delivered via external beam radiotherapy (EBRT), administering 78–80 Gy over 39–40 fractions, or, occasionally, via brachytherapy. When ADT was employed, it generally commenced two to three months before radiotherapy and involved luteinizing hormone-releasing hormone (LHRH) agonists, with or without anti-androgens.

Before radiotherapy and for a period ranging from six to thirty-six months thereafter, contingent upon the patient's risk stratification, the primary modality for evaluating therapeutic response was quantifying serum prostate-specific antigen (PSA) levels at baseline and subsequently at three-month intervals.

The threshold of 6.5 ng/mL was determined as a pragmatic, institution-specific cutoff based on the distribution of post-treatment PSA levels within the cohort, specifically representing the 75th percentile at

three months following definitive therapy. This value was selected before analysis to balance sensitivity and specificity in early biochemical response assessment, facilitating the prompt identification of patients with inadequate PSA decline who may require closer monitoring or earlier salvage therapy. It is important to note that this cutoff is non-standard and has not undergone external validation; traditional endpoints such as biochemical recurrence-free survival or the Phoenix criteria (PSA nadir + 2 ng/mL) are intended for long-term follow-up and were not suitable for the early (3-month) evaluation window. Therefore, the 6.5 ng/mL threshold should be interpreted as an exploratory, context-specific marker of response rather than a validated surrogate for long-term oncologic outcomes.

Responders were defined as those with a PSA decline below 6.5 ng/mL, a pivotal biochemical indicator of favorable response; conversely, individuals with higher PSA levels were categorized as non-responders. Complementary assessments, including digital rectal examination (DRE) and, when indicated, multiparametric magnetic resonance imaging (MRI), were employed to evaluate both local and systemic disease status. It is important to stress that this threshold was chosen solely for exploratory, hypothesis-generating analysis. It is not intended for clinical use, and all subsequent predictive analyses should be interpreted with the understanding that this data-driven cutoff has an inherent risk of overfitting.

2.4. Ethical Considerations:

The study protocol was approved by the Institutional Review Board and Ethics Committee of the University of Baghdad (IRB No. 5/23-2-2026), ensuring compliance with the ethical standards outlined in the 2013 revision of the Declaration of Helsinki. All participants provided written informed consent after receiving a thorough explanation of the study's objectives, procedures, potential risks, and benefits. To protect patient confidentiality, data were anonymized using unique identifiers. All clinical activities were carried out in accordance with applicable institutional and national guidelines.

2.5. Statistical Analysis

This prospective study, encompassing ninety patients diagnosed with prostate cancer, employed rigorous statistical methods. Baseline characteristics were examined using t-tests, Mann-Whitney U tests, and Chi-squared tests. Changes in biomarker concentrations over time (T0 vs. T1) were analyzed using the Wilcoxon signed-rank test. To address the biological heterogeneity arising from different therapeutic strategies, biomarker alterations (Δ) were categorized by treatment modality (radical prostatectomy (RP) or radiotherapy with or without androgen deprivation therapy (RT $\pm$ ADT)) and compared using the Mann-Whitney U test.

Additionally, a two-way repeated measures ANOVA was performed to assess the interaction between treatment type and time on biomarker levels. ROC analysis was utilized to assess the ability of individual biomarkers to differentiate outcomes. A multivariable logistic regression

model was developed to determine independent predictors of biochemical response, with adjustments made for potential confounders such as age, Gleason score (≤ 7 vs. ≥ 8), baseline PSA, and treatment modality (RP vs. RT $\pm$ ADT), to confirm that the type of intervention did not influence the biomarkers' predictive value. Furthermore, a Random Forest classifier was employed to evaluate the overall predictive performance of the biomarker panel. Spearman's rank correlation analysis was conducted to examine the relationships between biomarker levels and PSA changes. A p-value less than 0.05 was considered statistically significant for primary analyses [31].

Given the evaluation of six biomarkers across multiple comparisons, there is a potential, albeit minimized, risk of inflated Type I error. Nonetheless, the primary findings for SDF1, adenosine, IL-39, and LPS remained statistically significant after applying the conservative Bonferroni correction ($p < 0.0083$). As recommended for discovery-phase studies, we report exact p-values and effect sizes, emphasizing the need for external validation. Furthermore, RStudio was used to generate the figures [32].

3. Results

3.1. Baseline Patient Characteristics Stratified by Treatment Response:

The cohort comprised ninety patients diagnosed with either localized or locally advanced prostate adenocarcinoma. An early response marker was set as a post-treatment PSA level below 6.5 ng/mL at three months following definitive therapy. Utilizing this criterion, fifty-five patients (61.1%) were categorized as responders, whereas thirty-five patients (38.9%) were classified as non-responders. Demographic characteristics indicated that the mean age of the entire cohort was 65.3 years (SD = 7.8 years), with ages ranging from 51 to 82 years. Statistical analysis revealed no significant difference in age between responders (mean 64.8 years, SD 8.0) and non-responders (mean 66.1 years, SD 7.5).

Baseline PSA: Pre-treatment PSA levels exhibited a non-normal distribution (Shapiro-Wilk test, $p < 0.001$). The median baseline PSA for the cohort was 18.3 ng/mL (IQR: 13.2-37.8). While non-responders showed higher median baseline PSA values (22.6 ng/mL, IQR: 14.6 to 42.1) compared to responders (17.2 ng/mL, IQR: 12.1 to 35.7), this difference was not statistically significant (Mann-Whitney U test, $U = 812.5$, $p = 0.165$). A notable difference was observed in the distribution of tumor grades between the response groups. Among those who responded, 36 patients (65.5%) had a Gleason score of 7 or lower, whereas 19 patients (34.5%) had a score of 8 or higher. Conversely, non-responders showed a higher prevalence of high-grade tumors: 16 patients (45.7%) had a Gleason score of 8 or higher, and 19 patients (54.3%) had a score of 7 or lower. This difference was statistically significant (Pearson's Chi-squared test, $\chi^2 = 4.43$, $df = 1$, $p = 0.035$).

The distribution of clinical T-stage was similar across the groups. Specifically, 62 patients (68.9%) presented

with T2 disease, while 28 (31.1%) had T3 disease. Among responders, T2 was observed in 39 cases (70.9%) and T3 in 16 cases (29.1%). Non-responders showed T2 in 23 cases (65.7%) and T3 in 12 cases (34.3%). Statistical analysis using Pearson's Chi-squared test indicated no significant difference between the groups ($\chi^2 = 0.41$, $df = 1$, $p = 0.521$). In the context of primary definitive therapy, 41 patients (45.6%) underwent radical prostatectomy (RP), while 49 patients (54.4%) received radiotherapy, with or without androgen deprivation therapy (RT $\pm$ ADT). Among responders, 28 patients (50.9%) underwent RP, and 27 (49.1%) received RT $\pm$ ADT. Conversely, among non-responders, 13 patients (37.1%) underwent RP, whereas 22 (62.9%) received RT $\pm$ ADT. Although a greater proportion of responders chose surgical intervention, this difference was not statistically significant (Pearson's Chi-squared test, $\chi^2 = 2.68$, $df = 1$, $p = 0.102$) (Table 1).

3.2. Longitudinal Changes in Circulating Biomarkers Following Treatment

Paired comparisons of biomarker levels before (T0) and after (T1) treatment across the entire cohort ($n=90$) demonstrated notable systemic changes after definitive prostate cancer therapy. Four biomarkers showed marked increases in circulating concentrations three months post-treatment. Notably, the median level of Stromal Cell-Derived Factor 1 Alpha (SDF1) increased from 8.23 ng/mL [3.58, 33.71] at baseline to 60.80 ng/mL [49.54, 65.59] after treatment ($p < 0.001$). Similar significant elevations were observed for Interleukin-39 (IL-39), rising from 0.742 pg/mL [0.613, 1.173] to 1.695 pg/mL [1.401, 2.199]; GFRAL from 0.126 ng/mL [0.117, 0.143] to 0.341 ng/mL [0.328, 0.366]; and Lipopolysaccharide (LPS) from 1.704 ng/mL [1.324, 2.530] to 2.040 ng/mL [1.850, 2.790], all with p -values below 0.001.

In contrast, circulating adenosine (AD) levels experienced a significant reduction following treatment, decreasing from a median of 134.5 ng/mL [81.5, 274.9] to

101.7 ng/mL [60.2, 197.6] ($p < 0.001$). Conversely, levels of tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) did not demonstrate a statistically significant change post-therapy, with pre-treatment values at 0.254 ng/mL [0.212, 0.806] and post-treatment values at 0.119 ng/mL [0.113, 0.310] ($p = 0.415$). (Table 2)

3.3. Biomarker Profiles Differentiate Treatment Responders:

Post-treatment biomarker levels were analyzed to distinguish responders ($n=55$) from non-responders ($n=35$) and to identify profiles associated with biochemical response. The analysis demonstrated that responders exhibited a distinct biomarker signature compared to non-responders. Specifically, responders had significantly higher post-treatment levels of Stromal Cell-Derived Factor 1 (SDF1) (Responders: 63.9 [60.3, 65.6] ng/mL vs. non-responders: 45.6 [38.1, 60.8] ng/mL; $p < 0.001$) and Interleukin-39 (IL-39) (Responders: 1.784 [1.536, 2.199] pg/mL vs. non-responders: 1.401 [0.920, 1.931] pg/mL; $p = 0.004$). Conversely, responders showed significantly lower post-treatment levels of Adenosine (AD) (Responders: 87.1 [60.2, 197.6] ng/mL vs. non-responders: 197.1 [112.9, 286.0] ng/mL; $p = 0.003$) and Lipopolysaccharide (LPS) (Responders: 1.95 [1.85, 2.22] ng/mL vs. non-responders: 2.46 [2.04, 2.79] ng/mL; $p = 0.002$). No significant differences in post-treatment levels of GFRAL ($p=0.062$) and TRAIL ($p=0.415$) were observed between the two groups (Table 3, Figure 1).

Adenosine levels displayed substantial variability within groups, with wide interquartile ranges observed in both responders and non-responders. Factors such as tumor hypoxia, immune status, renal function, and undetected influences on metabolism may contribute. Despite heterogeneity, adenosine remained a significant predictor of response, highlighting its association with better biochemical outcomes post-treatment.

Notably, all significant differences between groups (SDF1, AD, IL-39, and LPS) remained statistically

Table 1. Baseline Clinical Characteristics of Prostate Cancer Patients Stratified by Treatment Response

Characteristic	Total (N=90)	Responders (n=55)	Non-Responders (n=35)	Test Statistic	p-value
Age, years (Mean $\pm$ SD)	65.3 $\pm$ 7.8	64.8 $\pm$ 8.0	66.1 $\pm$ 7.5	$t(88) = 0.80$ •	0.427
Baseline PSA, ng/mL (Median [IQR])	18.3 [13.2, 37.8]	17.2 [12.1, 35.7]	22.6 [14.6, 42.1]	$U = 812.5$ ‡	0.165
Gleason Score, n (%)				$\chi^2 = 4.43$ §	0.035
≤ 7	55 (61.1)	36 (65.5)	19 (54.3)		
≥ 8	35 (38.9)	19 (34.5)	16 (45.7)		
Clinical Stage, n (%)				$\chi^2 = 0.41$ §	0.521
T2	62 (68.9)	39 (70.9)	23 (65.7)		
T3	28 (31.1)	16 (29.1)	12 (34.3)		
Treatment Modality, n (%)				$\chi^2 = 2.68$ §	0.102
RP	41 (45.6)	28 (50.9)	13 (37.1)		
RT $\pm$ ADT	49 (54.4)	27 (49.1)	22 (62.9)		

Data are expressed as means with standard deviations (SD), medians accompanied by interquartile ranges (IQR), or counts with percentages in parentheses. Percentages may not sum to exactly 100% due to rounding. • Two-tailed independent samples t-test assuming equal variances (Levene's test, $p = 0.612$). ‡ Mann-Whitney U test (applied because of a non-normal distribution, assessed by the Shapiro-Wilk test, $p < 0.001$). § Pearson's Chi-squared test (with Yates' continuity correction applied where expected cell counts < 5). PSA, prostate-specific antigen; RP, radical prostatectomy; RT, radiotherapy; ADT, androgen deprivation therapy; SD, standard deviation; IQR, interquartile range; t, t-test statistic; U, Mann-Whitney U statistic; χ^2 , Chi-squared statistic; df, degrees of freedom.

Table 2. Circulating Biomarker Levels Before and After Treatment (n=90)

Biomarker	Pre-Treatment (T0) Median [IQR]	Post-Treatment (T1) Median [IQR]	p-value (Wilcoxon)
GFRAL (ng/mL)	0.126 [0.117, 0.143]	0.341 [0.328, 0.366]	<0.001
AD (ng/mL)	134.5 [81.5, 274.9]	101.7 [60.2, 197.6]	<0.001
IL-39 (pg/mL)	0.742 [0.613, 1.173]	1.695 [1.401, 2.199]	<0.001
TRAIL (ng/mL)	0.254 [0.212, 0.806]	0.119 [0.113, 0.310]	0.415
LPS (ng/mL)	1.704 [1.324, 2.530]	2.040 [1.850, 2.790]	<0.001
SDF1 (ng/mL)	8.23 [3.58, 33.71]	60.80 [49.54, 65.59]	<0.001

Data are expressed as medians with interquartile ranges (IQR). The Wilcoxon signed-rank test for paired samples was employed to compare biomarker levels before treatment (T0) and after treatment (T1). A p-value less than 0.05 was deemed statistically significant. AD, adenosine; LPS, Lipopolysaccharide; SDF1, Stromal cell-derived factor 1 alpha; TRAIL, TNF-related apoptosis-inducing ligand.

significant after applying the Bonferroni correction for multiple comparisons (adjusted threshold: $p < 0.0083$), thereby confirming the robustness of these findings against Type I error inflation.

To clarify the multidimensional differences among response groups based on six biomarkers, Principal Component Analysis (PCA) was conducted. As shown in Figure 2, the first two principal components (PC1 and PC2) accounted for 48% and 22% of the total variance, respectively, and together explained 70% of the overall variance in the post-treatment biomarker data. While this suggests that the two-dimensional projection represents a significant portion of the information in the six-dimensional biomarker space, it is important to recognize that 30% of the variance remains unaccounted for by PC1 and PC2, highlighting the complexity and multifaceted nature of systemic immunometabolic responses to prostate cancer treatment.

The PCA biplot demonstrates distinct clustering, with responder and non-responder groups primarily separated along the first principal component (PC1). Biomarker vectors indicate that SDF1, IL-39, AD, and LPS were the main contributors to this segregation, as evidenced by their high loadings on PC1 (SDF1: 0.84; IL-39: 0.71; AD: -0.68; LPS: -0.62), aligning with the univariate analysis. Figure 2.

3.4. Diagnostic Performance of Post-Treatment Biomarkers in Predicting Therapeutic Response:

To more comprehensively evaluate the predictive capabilities of the six circulating biomarkers, receiver operating characteristic (ROC) curves were constructed.

The efficacy of each biomarker in distinguishing responders from non-responders was evaluated. SDF1 demonstrated the most significant discriminatory ability,

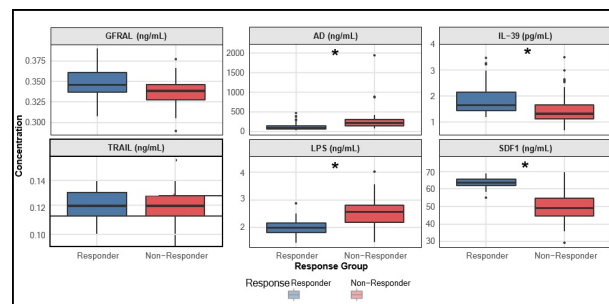


Figure 1. Post-Treatment Circulating Biomarker Levels in Biochemical Responders vs. Non-Responders. (This figure illustrates the distribution of six circulating immune and metabolic biomarkers measured three months following definitive prostate cancer therapy. The analyzed biomarkers include GFRAL (ng/mL), adenosine-AD (ng/mL), interleukin-39 (IL-39, pg/mL), TRAIL (ng/mL), lipopolysaccharide (LPS, ng/mL), and stromal cell-derived factor 1 (SDF1, ng/mL). Subjects are stratified based on biochemical response status: responders (blue, n=55; post-treatment PSA <6.5 ng/mL) and non-responders (red, n=35; PSA ≥6.5 ng/mL). Box plots depict the interquartile range (25th–75th percentiles), with horizontal lines indicating median values and whiskers extending to 1.5 times the interquartile range. Points represent individual outliers. Statistically significant differences between response groups are marked with asterisks: SDF1 ($p < 0.001$), IL-39 ($p = 0.004$), AD ($p = 0.003$), and LPS ($p = 0.002$). GFRAL ($p = 0.062$) and TRAIL ($p = 0.415$) did not show significant differences. Notably, responders demonstrated markedly higher levels of SDF1 and IL-39 and lower levels of AD and LPS than non-responders.

Table 3. The Biomarker Levels Between Responders and Non-Responders

Biomarker (Unit)	Responders (n=55) Median [IQR]	Non-Responders (n=35) Median [IQR]	p-value (Mann-Whitney U)
GFRAL (ng/mL)	0.347 [0.328, 0.366]	0.336 [0.319, 0.353]	0.062
AD (ng/mL)	87.1 [60.2, 197.6]	197.1 [112.9, 286.0]	0.003
IL-39 (pg/mL)	1.784 [1.536, 2.199]	1.401 [0.920, 1.931]	0.004
TRAIL (ng/mL)	0.119 [0.113, 0.138]	0.121 [0.110, 0.142]	0.415
LPS (ng/mL)	1.95 [1.85, 2.22]	2.46 [2.04, 2.79]	0.002
SDF1 (ng/mL)	63.9 [60.3, 65.6]	45.6 [38.1, 60.8]	<0.001

Data are expressed as the median with interquartile range (IQR) due to non-normal data distribution. Comparisons between groups were conducted using the Mann-Whitney U test. A p-value less than 0.05 was deemed statistically significant. Abbreviations: AD, adenosine; LPS, lipopolysaccharide; SDF1, stromal cell-derived factor 1 alpha; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand.

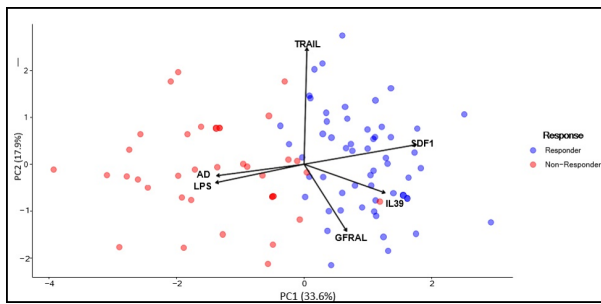


Figure 2. Principal Component Analysis (PCA) Biplot of Post-Treatment Circulating Biomarker Profiles by Biochemical Response Status. (Component analysis biplot depicting the multivariate biomarker profiles of prostate cancer patients three months post-therapy (n=90). Each point denotes an individual patient, color-coded by biochemical response: responders (blue, n=55; post-treatment PSA <6.5 ng/mL) and non-responders (red, n=35; PSA ≥6.5 ng/mL). Vectors indicate the direction and contribution of six circulating biomarkers to the first two principal components. The first principal component (PC1) accounts for 48% of the variance, while the second principal component (PC2) explains an additional 22%, resulting in a cumulative explained variance of. The separation between response groups is primarily along PC1, which is influenced by increased levels of SDF1 and IL-39 and decreased levels of adenosine (AD) and LPS in responders. It is important to recognize that 30% of the total variance remains unexplained by this two-dimensional analysis, suggesting that additional unmeasured factors contribute to shaping the systemic immunometabolic response phenotype. The clustering pattern observed supports the potential of a combined immune-metabolic biomarker signature for distinguishing patients who exhibit favorable biochemical responses from those with suboptimal outcomes after prostate cancer treatment.

with an area under the curve (AUC) of 0.89 (95% CI: 0.82–0.95, $p < 0.001$), followed by AD (AUC = 0.85, 95% CI: 0.77–0.92, $p < 0.001$). IL-39 and LPS exhibited moderate predictive performance, with AUCs of 0.76 and 0.73, respectively. GFRAL showed limited discriminative capacity (AUC = 0.62, $p = 0.034$), while TRAIL did not effectively differentiate between the groups (AUC = 0.51, $p = 0.841$).

A logistic regression model that incorporates the three most predictive biomarkers—SDF1, AD, and IL-39 exhibited enhanced diagnostic efficacy, achieving an area

under the curve (AUC) of 0.92 (95% CI: 0.86–0.97), with a sensitivity of 89.1%, specificity of 85.7%, and an overall accuracy of 87.8%. Table 4, Figure 3

3.5. Correlation with PSA and Predictive Modeling

To assess the relationship between post-treatment biomarker levels and the degree of therapeutic response, Spearman's rank correlation analysis was performed using the absolute change in PSA (Δ PSA), calculated as the difference between pre- and post-treatment PSA values. The analysis demonstrated distinct correlation patterns. A strong positive correlation was observed between Δ PSA and SDF1 ($\rho = 0.72$, $p < 0.001$), as well as IL-39 ($\rho = 0.51$, $p < 0.001$), indicating that greater declines in PSA are significantly associated with higher circulating levels of these immune-activating and tissue-repair mediators. Conversely, significant negative correlations were identified for adenosine (AD) ($\rho = -0.65$, $p < 0.001$) and LPS ($\rho = -0.47$, $p < 0.001$), suggesting that lower post-treatment concentrations of these immunosuppressive and pro-inflammatory markers are linked to more favorable biochemical responses. GFRAL and TRAIL did not show significant correlations with Δ PSA ($\rho = 0.18$, $p = 0.092$ and $\rho = -0.09$, $p = 0.410$, respectively).

To assess whether these biomarkers independently predicted biochemical response, a multivariate logistic regression model was created, including all six biomarkers along with key clinical confounders: age (continuous), Gleason score (≤ 7 vs. ≥ 8), baseline PSA (continuous), and treatment modality (radical prostatectomy versus radiotherapy with or without androgen deprivation therapy). The full model output, including β -coefficients, odds ratios, and confidence intervals for all variables, is shown in Table 5.

Biomarker Predictors: After thorough adjustment, two biomarkers maintained their independent predictive significance. Post-treatment SDF1 concentration was a strong positive predictor of response (OR: 1.08 per ng/mL; 95% CI: 1.04–1.12; β -coefficient: 0.077; $p < 0.001$), with each 1 ng/mL increase associated with an 8% higher chance of biochemical response. In contrast, adenosine (AD) remained a significant negative predictor (OR: 0.98 per ng/mL; 95% CI: 0.97–0.99; β -coefficient:

Table 4. Diagnostic Performance of Post-Treatment Biomarkers in Distinguishing Responders from Non-Responders

Biomarker	AUC (95% CI)	p-value	Optimal Cutoff	Sensitivity (%)	Specificity (%)
SDF1	0.89 (0.82–0.95)	<0.001	>58.4 ng/mL	85.5	82.9
AD	0.85 (0.77–0.92)	<0.001	≤95.2 ng/mL	80.0	77.1
IL-39	0.76 (0.66–0.85)	<0.001	>1.65 pg/mL	74.5	71.4
LPS	0.73 (0.62–0.83)	<0.001	≤2.15 ng/mL	69.1	68.6
GFRAL	0.62 (0.50–0.73)	0.034	>0.338 ng/mL	65.5	57.1
TRAIL	0.51 (0.39–0.63)	0.841	≤0.125 ng/mL	54.5	48.6
Combined Panel (SDF1 + AD + IL-39)	0.92 (0.86–0.97)	<0.001	—	89.1	85.7

shows the diagnostic performance of post-treatment circulating biomarkers in distinguishing biochemical responders (PSA < 6.5 ng/mL) from non-responders (PSA ≥ 6.5 ng/mL) using receiver operating characteristic (ROC) curve analysis. The combined panel was constructed using logistic regression incorporating SDF1, AD, and IL-39. AUC: area under the curve; CI: confidence interval. Optimal cutoffs were determined using the Youden index (J).

-0.020; $p = 0.002$), suggesting that lower post-treatment adenosine levels independently forecast a favorable outcome. Though IL-39 and LPS showed directional trends consistent with the univariate analysis, they did not retain independent predictive value after full adjustment ($p = 0.089$ and $p = 0.102$, respectively). GFRAL and TRAIL showed no significant links in the multivariate analysis ($p = 0.456$ and $p = 0.315$, respectively).

Clinical Predictors: Of the clinical variables included in the model, Gleason score was the only significant independent predictor (OR: 0.42 for Gleason ≥ 8 vs. ≤ 7 ; 95% CI: 0.18–0.98; β -coefficient: -0.867; $p = 0.045$), confirming that high-grade tumors are linked to lower odds of biochemical response even after accounting for biomarker profiles. Age ($p = 0.412$), baseline PSA ($p = 0.238$), and treatment modality ($p = 0.151$) did not reach statistical significance in the fully adjusted model, indicating that their prognostic effects are mediated by or overshadowed by biomarker and grade information.

The ongoing importance of SDF1 and adenosine as predictors even after rigorous statistical adjustments for treatment type and Gleason score emphasizes their robustness and biological significance. Notably, adding biomarkers to the model reduced the predictive power of baseline PSA and treatment modality, suggesting that these circulating markers reflect aspects of host response biology not captured by traditional clinical parameters. Successful prostate cancer treatments, regardless of method, seem to lead to a common biological endpoint marked by increased SDF1-driven tissue repair and decreased adenosine-mediated immunosuppression. (Table 5).

3.6. Stratification of Biomarker Changes by Treatment Modality:

To assess whether systemic immunometabolic

alterations were affected by treatment type and to confirm the reliability of our multivariate adjustments, biomarker changes were categorized by treatment: radical prostatectomy (RP, $n=41$) versus radiotherapy with or without androgen deprivation therapy (RT $\pm$ ADT, $n=49$). Initial biomarker levels did not differ significantly between groups, suggesting similar baseline systemic profiles.

Post-treatment analyses demonstrated distinct patterns in the magnitude of biochemical changes. Patients with RP showed significantly greater increases in SDF1, with a median change of +54.2 ng/mL, compared to +48.1 ng/mL in patients receiving RT with or without ADT ($p=0.018$). Conversely, patients undergoing RT $\pm$ ADT experienced a greater decrease in adenosine levels (-41.3 ng/mL versus -28.7 ng/mL; $p=0.032$). No significant differences were observed for IL-39, LPS, GFRAL, or TRAIL.

Despite variations in the magnitude of biomarker fluctuations, the overall directional trend remained consistently similar across both treatment modalities. Specifically, levels of SDF1, IL-39, GFRAL, and LPS increased, while adenosine decreased following both the RP and RT $\pm$ ADT protocols. A two-way repeated measures ANOVA revealed a significant main effect of time (pre- versus post-treatment) on these biomarkers (all $p<0.001$), with no significant interaction between time and treatment. These findings suggest that systemic immunometabolic reprogramming occurs independently of the specific treatment approach, with only modest differences in the extent of change.

Most notably, the biomarker signature that differentiates responders from non-responders characterized by elevated levels of SDF1 and IL-39 and reduced levels of adenosine and LPS was consistently observed across both treatment subgroups. This consistency reinforces the internal validity of the subsequent multivariable analysis, indicating that the identification of SDF1 and AD as independent

Table 5. Spearman Correlation and Multivariate Logistic Regression Analysis of Post-Treatment Biomarkers with PSA Response

Biomarker	Spearman's ρ with Δ PSA	p-value	Multivariate Logistic Regression ¹	p-value
SDF1	0.72	<0.001	OR: 1.08 per ng/mL (95% CI: 1.04–1.12)	<0.001
AD	-0.65	<0.001	OR: 0.98 per ng/mL (95% CI: 0.97–0.99)	0.002
IL-39	0.51	<0.001	OR: 1.02 per pg/mL (95% CI: 0.99–1.05)	0.089
LPS	-0.47	<0.001	OR: 0.96 per ng/mL (95% CI: 0.92–1.01)	0.102
GFRAL	0.18	0.092	OR: 1.01 per ng/mL (95% CI: 0.98–1.04)	0.456
TRAIL	-0.09	0.410	OR: 0.99 per ng/mL (95% CI: 0.97–1.01)	0.315
Clinical Variables				
Age (per year)	—	—	OR: 0.98 per year (95% CI: 0.94–1.03) β -coefficient: -0.020	0.412
Baseline PSA (per ng/mL)	—	—	OR: 1.01 per ng/mL (95% CI: 0.99–1.03) β -coefficient: 0.009	0.238
Gleason Score (≥ 8 vs. ≤ 7)	—	—	OR: 0.42 (95% CI: 0.18–0.98) β -coefficient: -0.867	0.045
Treatment Modality (RT $\pm$ ADT vs. RP)	—	—	OR: 0.51 (95% CI: 0.20–1.28) β -coefficient: -0.673	0.151

This multivariate model was adjusted for all variables listed. Statistically significant values are indicated in bold ($p<0.05$). Δ PSA represents the change in prostate-specific antigen, calculated as the difference between pre-treatment and post-treatment levels. OR denotes odds ratio, while CI stands for confidence interval. RP refers to radical prostatectomy, and RT $\pm$ ADT indicates radiotherapy with or without androgen deprivation therapy. AD is short for adenosine, LPS for lipopolysaccharide, SDF1 is stromal cell-derived factor 1 alpha, and TRAIL stands for tumor necrosis factor-related apoptosis-inducing ligand.

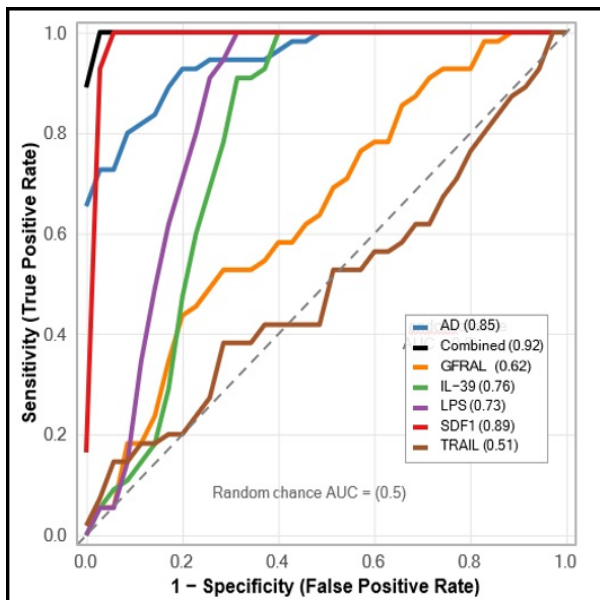


Figure 3. (ROC) Curves of Circulating Immune-Metabolic Biomarkers for Predicting Biochemical Response in Prostate Cancer Therapy. (Curves illustrating the diagnostic performance of six circulating immune and metabolic biomarkers in differentiating biochemical responders from non-responders after definitive prostate cancer therapy (n=90). Each biomarker curve is color-coded: SDF1 (red, AUC=0.89), adenosine (AD, blue, AUC=0.85), interleukin-39 (IL-39, green, AUC=0.76), lipopolysaccharide (LPS, purple, AUC=0.73), GFRAL (orange, AUC=0.62), and TRAIL (brown, AUC=0.51). The dashed black line denotes a three-biomarker combined panel (SDF1 + AD + IL-39) with superior predictive accuracy (AUC = 0.92). The diagonal gray dashed line represents the threshold of random chance (AUC=0.5). Responders were classified based on post-treatment PSA levels below 6.5 ng/mL, whereas non-responders exhibited PSA levels equal to or exceeding 6.5 ng/mL.

predictors is not an artifact of treatment selection bias, but rather a genuine reflection of host response. Table 6

4. Discussion

Managing prostate cancer presents ongoing challenges due to varied patient responses and the lack of reliable, non-invasive markers for treatment success. Conventional markers like PSA offer limited prognostic value, underscoring the need for advanced multi-marker panels to improve patient classification. This study employs a

targeted, multi-biomarker approach, analyzing circulating immune and metabolic analytes via standardized ELISA assays. The goal is to identify systemic signatures, elucidate biological pathways, and develop a refined hypothesis-generating predictive framework to support future personalized clinical decisions.

The study's baseline clinical characteristics revealed a significant difference in Gleason score distribution between responders and non-responders, with a higher proportion of non-responders exhibiting scores of 8 or above. This aligns with established oncological knowledge, as the Gleason score is a recognized marker of tumor aggressiveness [33]. Higher-grade tumors demonstrate more disorganized glandular architecture, increased cellular proliferation, and greater invasive and treatment-resistant tendencies. These features are likely driven by dysregulation of cellular pathways, enhanced survival signals, and an immunosuppressive, metabolically adaptable tumor microenvironment [34].

Interestingly, although baseline PSA levels and clinical T-stage showed no significant differences between groups, variation in Gleason score suggests that tumor biology is more influential than initial tumor size or PSA secretion alone in determining therapeutic outcomes. Tumor grade is pivotal in systemic response and biochemical trajectory [35-37]. The observed pattern, indicating differing treatment approaches with a higher rate of radical prostatectomy among responders, is likely affected by clinical selection bias, as surgeons favor surgery for patients with more favorable anatomy or less advanced disease [37, 38]. However, this trend did not reach statistical significance within this cohort.

Our longitudinal study revealed systemic changes in the immune-metabolic environment after prostate cancer treatment. Notably, SDF1, IL-39, GFRAL, and LPS levels increased post-treatment, while adenosine decreased. A distinct biomarker signature differentiated responders from non-responders, with responders showing higher SDF1 and IL-39, and lower adenosine and LPS.

The notable increase in SDF1 (CXCL12), a chemokine that recruits hematopoietic and stromal progenitors, likely indicates a coordinated response to tissue repair and immune reconstitution after surgery or radiotherapy. Its strong correlation with PSA decline suggests SDF1/CXCR4 signaling could serve as a marker for tumor microenvironment remodeling and immune response [39, 40]. Conversely, the observed significant reduction in adenosine, a well-established immunosuppressive

Table 6. Comparison of Biomarker Changes ($\Delta = T1 - T0$) Stratified by Treatment Modality

Biomarker (Unit)	RP (n=41) Median Δ [IQR]	RT $\pm$ ADT (n=49) Median Δ [IQR]	p-value
SDF1 (ng/mL)	+54.2 [+48.7, +59.3]	+48.1 [+42.5, +53.8]	0.018
AD (ng/mL)	-28.7 [-51.4, -15.3]	-41.3 [-68.2, -22.1]	0.032
IL-39 (pg/mL)	+0.98 [+0.76, +1.21]	+0.91 [+0.69, +1.15]	0.214
LPS (ng/mL)	+0.31 [+0.18, +0.52]	+0.36 [+0.21, +0.58]	0.187
GFRAL (ng/mL)	+0.21 [+0.19, +0.24]	+0.20 [+0.17, +0.23]	0.342
TRAIL (ng/mL)	-0.12 [-0.45, +0.08]	-0.14 [-0.51, +0.06]	0.456

Mann-Whitney U test comparing median change (Δ) between the RP and RT $\pm$ ADT groups. RP, radical prostatectomy; RT $\pm$ ADT, radiotherapy with or without androgen deprivation therapy; Δ , post-treatment minus pre-treatment value; IQR, interquartile range.

metabolite that inhibits T-cell and NK-cell activity, indicates a favorable shift from an immunologically “cold,” adenosine-rich tumor microenvironment, potentially facilitating the restoration of immune surveillance [41]. The elevation of IL-39, an interleukin with emerging functions in regulating inflammatory responses, and its correlation with the response may indicate a specific, advantageous T-helper cell polarization [42, 43].

The increase of IL-39 in responders presents a new, mechanistically significant aspect of the immunometabolic profile associated with effective prostate cancer treatment. IL-39 is a heterodimeric cytokine related to inflammation regulation [44]. While its role in prostate cancer is not well understood, IL-39 may influence T-helper cell polarization, promoting Th1 and Th17 responses, which are crucial for anti-tumor immunity post-treatment [45]. Alternatively, IL-39 may serve as a compensatory anti-inflammatory agent, reducing therapy-induced tissue damage while maintaining immune surveillance crucial for sustained remission [46]. The positive correlation between IL-39 and PSA decline suggests that IL-39 likely plays a role in effective immunologic recovery, rather than merely an epiphenomenon.

Furthermore, decreased LPS levels in responders highlight a different yet significant mechanism, with LPS indicating gut microbial translocation and systemic inflammation [47]. Our findings indicate that successful prostate cancer treatment correlates with reduced post-treatment LPS levels, suggesting restored intestinal barrier function and decreased microbiota-driven systemic inflammation, possibly through cytokine reduction or androgen deprivation effects [36, 48, 49]. Emerging evidence suggests gut dysbiosis and LPS-induced TLR4 activation contribute to resistant prostate cancer via NF- κ B and IL6/STAT3 pathways [50]. Thus, Lower LPS levels in responders may indicate reduced systemic inflammation and disruption of a pro-tumorigenic, therapy-resistant circuit. This highlights the role of the host-microbiome-immune axis in influencing therapeutic outcomes in prostate cancer. This signature demonstrates that effective therapy not only diminishes tumor burden but also triggers systemic reprogramming of key immune and metabolic pathways. It shifts from a suppressive environment with high adenosine and LPS to an active, reparative state characterized by elevated SDF1 and IL-39 levels, capturing dynamic biological processes beyond static snapshots.

A key finding supporting the biological importance of our 6.5 ng/mL cutoff is the clear and consistent immunometabolic profile observed in groups divided by this threshold. This statistically derived cutoff effectively separated patients into two distinct biological states (responders with high SDF1/IL-39 and low adenosine/LPS, and non-responders with the opposite pattern), underscoring its biological relevance. Such clear differentiation is unlikely to be due to chance. The alignment of immune and metabolic markers, involved in tissue repair, immunosuppression, and inflammation, with the PSA groups provides strong internal validation. This suggests that a PSA of 6.5 ng/mL at three months

signifies two different systemic biological phenotypes: one supporting tumor control and recovery, the other associated with immunosuppression and inflammation.

A critical consideration in biomarker studies involving heterogeneous oncologic cohorts is the potential confounding effect of disparate treatment modalities. Radical prostatectomy and radiotherapy with or without androgen deprivation exert fundamentally distinct local and systemic effects; surgery induces acute tissue injury and rapid antigen release, whereas radiotherapy promotes sustained immunogenic cell death over a longer temporal window [51-53]. To address this, biomarker kinetics were systematically stratified by treatment type. Significant differences were observed in the magnitude of SDF1 elevation and adenosine reduction between the RP and RT $\pm$ ADT groups; however, the direction of these changes was consistent across groups. A two-way repeated-measures ANOVA indicated no significant interaction between time and treatment, suggesting that post-therapeutic immunometabolic reprogramming represents a broad host response rather than a modality-specific effect. Treatment modality was included as a covariate. Notably, SDF1 and adenosine maintained independent predictive value after adjustment, confirming their role as genuine biomarkers of biological response rather than treatment proxies. These methodological considerations support the robustness of our proposed signature for clinical application.

Our assessment of the diagnostic efficacy of biochemical markers through ROC analysis indicated that post-treatment SDF1 exhibited the highest individual predictive accuracy for identifying biochemical responders, followed by adenosine. The inclusion of SDF1, adenosine, and IL-39 in a logistic regression model further improved predictive performance, reaching an AUC of 0.92. This finding likely underscores the critical roles of these analytes in regulating the tumor microenvironment and in immune-metabolic reprogramming following therapy.

Analysis indicated that the extent of PSA decline (Δ PSA) was significantly linked to specific post-treatment biomarker changes. A strong positive correlation was observed between increased levels of SDF1 and IL-39, whereas adenosine and LPS showed a strong negative correlation. Treatment modality, along with SDF1 and adenosine after therapy, independently predicted biochemical response when controlling for confounders. These findings suggest a mechanistic link between immune-metabolic shifts and clinical outcomes. The correlation between SDF1 and PSA reduction highlights SDF1's potential as a surrogate marker of post-treatment tissue repair and immune activation, reflecting its roles in lymphocyte recruitment and stromal remodeling [54]. Conversely, the independent predictive significance of reduced adenosine concentrations further substantiates the hypothesis that disrupting an immunosuppressive tumor microenvironment, characterized by elevated adenosine levels, is an essential prerequisite for achieving a profound biochemical response [55]. Together, these findings substantiate the view that reprogramming of systemic biomarkers is not merely a secondary phenomenon but is fundamentally linked to and indicative of therapy's

effectiveness.

A key aspect of our study was the use of a data-driven PSA threshold of 6.5 ng/mL to define response, which may introduce overfitting and optimism bias. To mitigate this, we validated our findings through multiple internal methods. Unsupervised principal component analysis (PCA) revealed that biomarker data naturally clustered patients into groups consistent with our response classification, indicating inherent discriminative ability beyond the cutoff. Furthermore, significant graded correlations between biomarkers and the continuous measure of PSA change (Δ PSA) supported a biological dose-response relationship. The signature's predictive accuracy remained robust after adjusting for confounders such as Gleason score and treatment type.

This single-center study involving 90 Iraqi participants has limitations. The identified cutoff values particularly SDF1 >58.4 ng/mL and adenosine $\leq$ 95.2 ng/mL are specific to this cohort and may not be applicable elsewhere without further validation. These thresholds, derived from ROC analysis using the Youden index, carry a risk of overfitting. The key finding is a consistent pattern: responders tend to have higher SDF1 and IL-39 levels and lower adenosine and LPS levels. While internal validation supports these results, external validation in larger, multi-center studies is essential before clinical application.

This study recognizes several limitations. Conducted at a single institution with 90 patients and focusing on localized disease, the results may not be broadly generalizable. Although our key findings (SDF1, AD, IL-39, LPS) remained statistically significant after Bonferroni correction ($p < 0.0083$), the potential for Type I error due to multiple comparisons cannot be entirely excluded in this preliminary study. Moreover, while the multi-biomarker panel shows promise, a notable limitation is our reliance on a non-standard, data-driven PSA threshold (6.5 ng/mL) to define treatment response. Since this cutoff was derived from our specific cohort, it introduces the risk of overfitting and optimistic bias, potentially overstating the model's predictive accuracy, as reflected in the reported AUC of 0.92. Although internal validation methods, including unsupervised learning and multivariate analysis, support the findings, they cannot fully eliminate the inherent biases of exploratory research. Biological validation aligns with the immunometabolic profiles associated with this threshold, but its correlation with long-term clinical outcomes such as recurrence-free survival, metastasis, or mortality remains unconfirmed. Future studies are needed to validate this biomarker in larger, multicenter cohorts with extended follow-up.

In conclusion, prostate cancer management faces significant challenges due to the heterogeneity of patient responses to definitive therapies and the limited prognostic value of traditional biomarkers such as prostate-specific antigen (PSA). This variability highlights a critical need for more reliable, non-invasive tools to predict therapeutic outcomes and elucidate underlying biological response mechanisms accurately. Our prospective study directly addressed this gap by systematically profiling a panel of six circulating immune and metabolic biomarkers before

and after treatment in patients with localized prostate cancer.

The results reveal a unique post-treatment immunometabolic signature characterized by increased levels of SDF1 and IL-39, along with decreased levels of adenosine and LPS, that effectively distinguishes biochemical responders from non-responders. SDF1 emerges as the strongest individual predictor, with an AUC of 0.89, while a combined model including SDF1, adenosine, and IL-39 reaches an improved AUC of 0.92. These biomarkers exhibit significant correlations with PSA reduction and retain their predictive power independent of other clinical factors, suggesting that successful therapy triggers systemic reprogramming from an immunosuppressive, adenosine-rich state to a reparative, immune-active environment. This shift appears crucial for achieving a positive biochemical response, indicating an active role in the response mechanism beyond simple association.

Furthermore, the study shows that a consistent immunometabolic signature across surgery and radiotherapy signifies a fundamental biological reprogramming of the host response, regardless of treatment method. The 6.5 ng/mL PSA threshold, though exploratory, effectively differentiated two systemic biological phenotypes, supporting its potential as a hypothesis-generating tool. However, the link between this early biochemical classification and long-term clinical outcomes, such as biochemical recurrence-free or metastasis-free survival, remains unproven. Despite thorough internal validation using various analytical methods unsupervised learning, correlation analyses, treatment stratification, and multivariate adjustment the reliance on a single-center cohort and endpoint limits its broader applicability. External validation in an independent, multi-center cohort against long-term clinical outcomes, such as biochemical recurrence-free survival, metastasis-free survival, and cancer-specific mortality, is essential before integrating this panel or its threshold values into clinical practice. The reported cutoff values (SDF1 >58.4 ng/mL, adenosine $\leq$ 95.2 ng/mL) are preliminary and need confirmation across diverse populations, with this study serving as a foundation for future validation.

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Acknowledgements

The authors are truly thankful to the patients and their families for their invaluable participation and cooperation in this study. We deeply appreciate the nursing staff and clinical research coordinators at the Oncology Teaching Hospital in Baghdad for their dedicated support in patient care and sample collection. Our sincere thanks also go to the laboratory technicians for their meticulous work in sample processing and biomarker analysis.

Financial support and sponsorship

Self- funded.

Conflicts of interest

The authors declare no conflicts of interest.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors clarify that no generative AI or AI-assisted tools were utilized in this work.

Abbreviations

AD: Adenosine, ADT: Androgen Deprivation Therapy, AUC: Area Under the Curve, CI: Confidence Interval, CXCL12: C-X-C Motif Chemokine Ligand 12 (also known as SDF1), DRE: Digital Rectal Examination, EBRT: External Beam Radiotherapy, ELISA: Enzyme-Linked Immunosorbent Assay, GFRAL: GDNF Family Receptor Alpha-Like, Gy: Gray (unit of radiation dose), IL-39: Interleukin-39, IQR: Interquartile Range, LHRH: Luteinizing Hormone-Releasing Hormone, LPS: Lipopolysaccharide, ML: Machine Learning, MRI: Magnetic Resonance Imaging, NK-cell: Natural Killer Cell, OR: Odds Ratio, PCA: Principal Component Analysis, PSA: Prostate-Specific Antigen, R&D: Research and Development, ROC: Receiver Operating Characteristic, RP: Radical Prostatectomy, RT: Radiotherapy, SD: Standard Deviation, SDF1: Stromal Cell-Derived Factor 1 Alpha (also known as CXCL12), TME: Tumor Microenvironment, TNF: Tumor Necrosis Factor, TRAIL: TNF-Related Apoptosis-Inducing Ligand.

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