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A Semen Panel Signature Comprising sTWEAK Cytokine Levels and Expression of exomiR-221-3p and exomiR-222-3p for Assessing Prostate Cancer Prognosis, Diagnostic Utility, and Detection of Upgrading During Active Surveillance

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Study Need and Importance: Noninvasive biomarkers are urgently needed to improve prostate cancer (PCa) diagnosis, predict tumor aggressiveness, and guide management decisions, especially during active surveillance (AS). Although urine and serum-based tests exist, semen remains underutilized despite its biological relevance. This study evaluated a semen-based biomarker panel composed by sTWEAK, exomiR-221-3p, and exomiR-222-3p for its utility in PCa diagnosis, aggressiveness stratification (International Society of Urological Pathology grade), biochemical recurrence prediction, and upgrading detection in AS.

What We Found: Semen samples were obtained from 136 PCa patients (85 low-risk, 51 high-risk), 20 healthy controls, 35 biopsy-negative, and 51 AS patients. Levels of sTWEAK and exomiR-221-3p identified high-risk PCa with 88% accuracy (AUC = 0.937). sTWEAK alone distinguished PCa from controls (AUC = 0.897), outperforming PSA (AUC = 0.598). In AS patients, a model combining sTWEAK and the percentage of positive biopsy cores predicted upgrading with AUC = 0.824, outperforming classical clinical variables (PSA density + percent of positive cores + nuclear magnetic resonance). For biochemical recurrence, sTWEAK and PSA density formed a decision-tree model that stratified recurrence risk.

Limitations: Biomarker thresholds remain exploratory and require validation in larger, multicenter studies. Variability in semen quality across age and health conditions may affect reproducibility. Integration with imaging (eg, multiparametric MRI) and standardized clinical workflows is yet to be defined.

Interpretation for Patient Care: This semen-based biomarker panel offers a promising, cost-effective, and noninvasive strategy for PCa management. It may enhance diagnostic precision, reduce unnecessary biopsies, and guide risk-adapted treatment. If validated, its use could improve personalized care by supporting more accurate clinical decision-making in PCa management.

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A Semen Panel Signature Comprising sTWEAK Cytokine Levels and Expression of exomiR-221-3p and exomiR-222-3p for Assessing Prostate Cancer Prognosis, Diagnostic Utility, and Detection of Upgrading During Active Surveillance

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Purpose: We evaluated the seminal biomarker signature (sTWEAK, exomiR-221-3p, and exomiR-222-3p) in several prostate cancer (PCa) settings: diagnosis, tumor aggressiveness prognosis (International Society of Urological Pathology [ISUP] grade in radical prostatectomy specimens and biochemical recurrence [BCR]), and identifying biopsy upgrading in confirmatory biopsies during active surveillance (AS).

Materials and Methods: Semen samples were collected from 136 PCa patients classified by prostatectomy specimen ISUP grades and classified as low-risk

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Ethics Statement: This study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board Ethics Committee of Institut d'Investigació Sanitària Pere Virgili (Ref. CEIM205/2020). All participants provided written consent before starting the study.

Author Contributions:

Conception and design: Altuna-Coy, Alves-Santiago, Chacón, Ruiz-Plazas.
Data analysis and interpretation: Altuna-Coy, Ascaso-Til, Garcia-Fontgivell, Segarra-Tomás, Chacón, López-Ribelles, Arreaza-Gil, Ruiz-Plazas.
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Critical revision of the manuscript for scientific and factual content: Altuna-Coy, Alves-Santiago, Ruiz-Plazas, Chacón.
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Data Availability: All data generated or analyzed during this study are included in this article, and its additional information files. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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(ISUP I-II, n = 85) and high-risk patients (ISUP ≥III, n = 51), along with 20 healthy controls, 35 patients with negative biopsies, and 51 low-risk PCa patients under AS. The relative expression levels of exomiR-221-3p and exomiR-222-3p in semen exovesicles were measured. ELISA was used to quantify sTWEAK levels. Statistical analyses, including multivariable binary logistic, Cox multivariate regression analysis, Survival classification regression tree models, and ROC, among others, were used to evaluate the predictive value of the biomarkers.

Results: A model combining sTWEAK and exomiR-221-3p predicted high-risk PCa (AUC = 0.937), showing a linear biomarker-probability relationship but no clear cutoff. A model comprising PSA density and sTWEAK could be used for predicting BCR. sTWEAK alone distinguished PCa cases (AUC = 0.897). In AS patients, sTWEAK levels plus the percentage of positive biopsy cores best predicted upgrading (AUC = 0.824).

Conclusions: Incorporating sTWEAK and exomiR-221-3p, along with clinical parameters, offers a promising diagnostic panel for PCa and serves as a useful predictive tool for AS upgrading and BCR prognosis.

Key Words: semen, diagnosis, prognosis, active surveillance

Prostate cancer (PCa) is the most diagnosed cancer and the second leading cause of cancer-related death in men.[1] Prostate biopsy, guided by transrectal ultrasound,[2] remains the standard for detecting aggressive PCa based on elevated PSA levels or abnormal exams, with nuclear magnetic resonance (NMR) increasingly used to target suspicious areas.[3] However, 10% to 15% of high-grade PCa cases are missed, highlighting the limitations of current prognostic tools, leading to overtreatment of indolent PCa and undertreatment of aggressive tumors.

Many newly diagnosed PCa patients have early-stage indolent disease. Active surveillance (AS) helps reduce overtreatment by delaying or avoiding radical treatments and their associated morbidities. Although most low-risk PCa patients can be safely managed on AS,[4] many still opt for therapies such as surgery or radiation.[5] A consensus on optimal selection criteria and monitoring schedules for AS is lacking, requiring frequent patient assessment to minimize misclassification and progression.[6]

Various biomarkers are being incorporated into clinical care to aid in risk stratification for AS and treatment decisions.[7] Current biomarkers, such as those derived from tissue-based transcriptomic gene panels[8-10] and liquid biopsies,[11] provide some utility but are often not specific enough to replace PSA testing. Many also lack sensitivity and specificity or are expensive, underscoring the need for more precise and cost-effective tools to identify aggressive tumors early.[12]

Semen represents a promising biofluid for biomarker discovery due to its noninvasive collection and minimal patient discomfort. Although the prevalence of PCa increases with age in parallel with ejaculatory dysfunction, this condition does not affect all patients. The incidence of ejaculatory dysfunction ranges from 3% in patients aged 50 to 54 years to 35% in individuals aged 70 to 78 years.[13] Patients undergoing radical treatments for PCa usually have a life expectancy over 10 years and no severe comorbidities, making sample collection generally feasible. Since 40% of semen comes from prostatic tissue, it likely contains PCa biomarkers. Seminal plasma, rich in exovesicles with microRNAs, enhances its potential for disease detection.[14] Our research on semen revealed that high-risk PCa patients had lower sTWEAK levels and higher exomiR-221-3p and exomiR-222-3p expression compared with low-risk patients.[15]

This study evaluates a seminal biomarker panel for improving PCa diagnosis and prognosis (based on the International Society of Urological Pathology [ISUP] grade of the prostatectomy specimen and biochemical recurrence [BCR] probability) and its utility in predicting upgrading in AS patients to enhance risk stratification and treatment decisions.

MATERIALS AND METHODS

Prognostic Cohort

This cohort included 136 PCa patients who underwent RP at University Hospital Joan XXIII (HJ23; Tarragona, Spain) between 2019 and 2022. Patients were classified into low-risk (ISUP I and II, n = 85) and high-risk (ISUP ≥ III, n = 51) groups based on the 2014 ISUP-Gleason Grade (GG) and TNM classifications.[16,17] Patient characteristics are presented in Table 1.

BCR data were available for 122 of 136 patients. Of these 122 patients, 17 experienced BCR within 1 year, 22 within 2 years, and 26 within 3 years (Table 1). BCR was defined as a confirmed and progressive serum PSA level > 0.2 ng/mL after RP. Time zero for follow-up was defined as the date of RP. PSA measurements were initiated approximately 3 months postsurgery, and patients were followed until either BCR occurred or the last PSA measurement without evidence of recurrence, which served as the censoring date. Censoring occurred in cases of loss to follow-up, study completion without BCR, or death from non–PCa-related causes.

Inclusion criteria: Patients were included if they had a RP (risk assessment) or RP with follow-up (BCR). Patients were excluded if they had a history of other cancers, were older than 75 years, or had received prior treatment for PCa.

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Table 1. *Clinical and Pathological Characteristics of Prognostic Cohort*

Clinical profile of patients	Prognostic cohort				*P* value
	Low risk (n = 85)		High risk (n = 51)		
Age (y)	66	(61-69)	67	(62-70)	.061
BMI (kg/m^2)	27.96	(25.82-29.73)	26.99	(24.67-29.4)	.164
Prostatic volume (cc)	51.5	(37.8-70.5)	41.0	(32.0-52.0)	.077
Serum PSA levels (ng/mL)	6.30	(4.89-8.30)	6.86	(5.67-11.35)	.049
Serum dPSA levels	0.13	(0.09-0.2)	0.18	(0.12-0.3)	.137
% positive biopsy cores	20.0	(8.3-33.3)	38.1	(16.7-58.3)	< .001
ISUP-GG prostatectomy, No. (%)					
Low risk					
ISUP 1	45	(52.9)	-		< .001
ISUP 2	40	(47.1)	-		
High risk					
ISUP 3	—		28	(54.9)	
ISUP 4	—		12	(23.5)	
ISUP 5	—		11	(21.6)	
T pathological stage, No. (%)					
T2a	11	(12.9)	2	(3.9)	< .001
T2b	13	(15.4)	1	(2.0)	
T2c	49	(57.6)	21	(41.2)	
T3a	9	(10.6)	12	(23.5)	
T3b	3	(3.5)	15	(29.4)	
N pathological stage, NX or N0, No. (%)	85	(100)	45	(88.2)	< .001
ISUP-GG diagnostic biopsy, No. (%)					
Low risk					< .001
ISUP 1	55	(64.7)	6	(11.8)	
ISUP 2	23	(27.1)	15	(29.4)	
High risk					
ISUP 3	5	(5.9)	11	(21.6)	
ISUP 4	2	(2.3)	12	(23.5)	
ISUP 5	—		7	(13.7)	
T clinical stage, No. (%)					
T1c	70	(82.4)	29	(56.9)	.004
T2a	11	(12.9)	14	(27.5)	
T2b	4	(4.7)	6	(11.8)	
T2c	—		1	(2.0)	
T3	—		1	(2.0)	
N clinical stage, NX or N0, No. (%)	85	(100)	50	(98.0)	.284
M clinical stage, MX or M0, No. (%)	85	(100)	50	(98.0)	.284
Follow-up time (mo)	29.0	(14.5-56.5)	14.0	(7.5-32.0)	.001
BCR (y)					
<1	5		12		
<2	6		16		
<3	8		18		
sTWEAK in semen (pg sTWEAK/mg total semen protein)	1106	(684-1513)	824	(406-1184)	.011
ExomiR-221-3p (relative expression)	0.58	(0.15-1.13)	1.08	(0.48-1.50)	.004
ExomiR-222-3p (relative expression)	1.27	(0.55-3.13)	2.19	(1.18-4)	.033

Abbreviations: BCR, biochemical recurrence; dPSA, PSA density; ISUP, International Society of Urological Pathology; ISUP-GG, ISUP Grade Groups based on the Gleason score as follows: Gleason score ≤6 group I, 3 + 4 = 7 group II, 4 + 3 = 7 group III, 4 + 4 = 8 group IV, and 9-10 group V; M, metastasis stage; N, lymph node category; T stage, tumor category. Values are expressed as the median and IQR (p25; p75) where applicable.

Diagnostic Cohort

We recruited 20 healthy patients with PSA levels < 3.5 ng/mL and normal digital rectal examinations and 35 patients with PSA levels > 3.5 ng/mL and negative biopsies (Table 2). A PSA threshold of 3.5 ng/mL was used to enhance sensitivity for clinically significant PCa, consistent with previous

Table 2. *Clinical and Pathological Characteristics of the Diagnostic Cohort*

Clinical profile of patients	Diagnostic cohort						*P* value
	Healthy controls (n = 20)		Negative biopsy (n = 35)		PCa (positive biopsy; n = 136)		
Age (y)	66	(60-73)	65	(59-72)	66	(61-69)	.793
BMI (kg/m^2)	27.12	(25.32-29.65)	27.76	(24.34-31.54)	27.72	(25.03-29.41)	.910
Serum PSA levels (ng/mL)	1.13	(0.65-2.15)	5.83	(4.34-7.98)[a]	6.78	(5.00-8.79)[a]	< .001
sTWEAK in semen (pg sTWEAK/mg total semen protein)	2101	(1110-3255)	1540	(788-3241)	1007	(594-1678)	< .001
ExomiR-221-3p (relative expression)	0.28	(0.11-0.65)	0.64	(0.18-1.49)	0.70	(0.22-1.44)	.021
ExomiR-222-3p (relative expression)	1.61	(0.02-3.15)	1.03	(0.22-2.33)	1.50	(0.59-3.22)	.274

Abbreviation: PCa, prostate cancer.
Values are expressed as the median and IQR (p25; p75).
[a] Significant differences when comparing with healthy controls.

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Table 3. *Clinical and Pathological Characteristics of the Active Surveillance Cohort*

	AS cohort		
Clinical profile of patients	No upgrade (ISUP = 1; n = 33)	Upgrade (ISUP >1; n = 18)	*P* value
Age (y)	66 (61-73)	65.5 (58.5-74.0)	.898
BMI (kg/m^2)	28.26 (25.95-30.31)	26.26 (25.06-28.97)	.110
Prostatic volume (cc)	51.0 (43.5-74.3)	44.0 (30.0-60.5)	.060
Serum PSA levels (ng/mL)	5.66 (4.62-6.37)	6.53 (4.84-9.24)	.079
Serum dPSA levels	0.10 (0.07-0.14)	0.15 (0.12-0.21)	.002
% positive biopsy cores	10.0 (6.7-18.2)	17.4 (15.7-33.3)	.013
NMR, negative, No. (%)	9 (27.3)	2 (11.1)	.066
ISUP-GG diagnostic biopsy, ISUP 1, No. (%)	33 (100)	18 (100)	1.000
T clinical stage, No. (%)			
T1c	32 (97.0)	15 (83.3)	.027
T2a	1 (3.0)	3 (16.7)	
N clinical stage, NX or N0, No. (%)	33 (100)	18 (100)	1.000
M clinical stage, MX or M0, No. (%)	33 (100)	18 (100)	1.000
ISUP-GG confirmation biopsy, No. (%)			
ISUP 1	33 (100)	—	< .001
ISUP 2	—	13 (72.2)	
ISUP 3	—	3 (16.7)	
ISUP 4	—	2 (11.1)	
sTWEAK in semen (pg sTWEAK/mg total semen protein)	2282 (1991-3166)	1035 (606-1735)	< .001
ExomiR-221-3p (relative expression)	0.45 (0.27-0.77)	1.49 (0.20-2.83)	.047
ExomiR-222-3p (relative expression)	0.79 (0.240-2.81)	2.80 (0.76-4.91)	.044

Abbreviations: AS, active surveillance; dPSA, PSA density; ISUP, International Society of Urological Pathology; ISUP-GG, ISUP Grade Groups based on the Gleason score as follows: Gleason score ≤ 6 group I, $3+4=7$ group II, $4+3=7$ group III, $4+4=8$ group IV, and 9-10 group V; M, metastasis stage; N, lymph node category; NMR, nuclear magnetic resonance; T stage, tumor category.
Values are expressed as the median and IQR (p25; p75) where applicable.

studies.[18] For comparison, we included a prognostic cohort with PCa diagnosed by positive biopsy (n = 136).

AS Cohort

Fifty-one low-risk patients, based on EAU risk groups, were included in AS. Six months after the diagnostic biopsy, a second biopsy reclassified patients as no upgrade (ISUP I, n = 33) or upgrade (ISUP > I, n = 18). All patients had NMR before the confirmatory biopsy. Patient characteristics are presented in Table 3.

Participants gave written informed consent. The study was approved by the local ethics committee and conducted in accordance with the Declaration of Helsinki, Biomedical Research Law 14/2007, Royal Decree 1716/2011 on Biobanks, Organic Law 3/2018 on Personal Data Protection, and Ethical Committee guidelines (CEIm Ref. CEim171/2017 and CEim171/2020). Clinical parameters and tumor aggressiveness were documented.

Semen Sample Collection and Processing

In the prognostic and AS cohorts, samples were collected after the diagnostic biopsy, while in the diagnostic cohort, they were taken before. All samples were stored at 4°C, transferred to our biobank, centrifuged at 2000× *g* for 15 minutes, and stored at −80°C in the IISPV BioBanc (B.0000853 + B.0000854), part of the Spanish National Biobanks Platform (PT13/0010/0029 and PT13/0010/0062).

Semen Extracellular Vesicles Extraction and exomiRNA Quantification by Real-Time PCR

ExomiRNAs were isolated from 200 µL semen using the exoRNeasy Kit (Qiagen, Spain) and reverse transcribed with the miRCURY LNA Universal RT Kit (Exiqon, Spain). The expression of exomiR-221-3p and 222-3p was analyzed in duplicate on a 7900HT Fast PCR System, with normalization using exomiR-423-5p, exomiR-29a-5p, exomiR-497-5p, and exomiR-663b. Data were analyzed using the $2^{-\Delta\Delta Ct}$ method, excluding Ct values > 35.

ELISA

sTWEAK levels in semen were measured by ELISA (R&D Systems, the United Kingdom) and normalized to total protein using the BCA method.[19]

Statistical Analysis

Sample sizes were calculated using G*Power 3.1.9.7 to assess differences in exomiR-221-3p, exomiR-222-3p, and sTWEAK across clinical scenarios (risk assessment, diagnosis, and AS). Assuming a 2-fold change, equal SDs, > 80% power, and a 10% type 1 error rate, sizes were determined using the Wilcoxon-Mann-Whitney test. For risk assessment, a 1-tailed test required a minimum of 21 patients per group (low- vs high-risk). For diagnosis and AS, a 2-tailed comparison required at least 10 patients per group and 19 for subgroup comparisons.

Clinical and pathological data are presented as medians and IQR. Group comparisons used the Mann-Whitney *U* test (2 groups) and Kruskal-Walli test (3 groups).

BCR was analyzed as a time-to-event outcome using Cox regression to estimate HRs with 95% CIs, adjusting for confounders. Proportional hazards were verified with Schoenfeld residuals.

Classification and regression tree (CART) models were constructed. For diagnostic cohort, 70% of the data were used for training and 30% for testing with 10-fold cross-validation. For AS, due to small sample sizes, the full data set was used. Tree depth was limited to 3, and splits were based on the Gini impurity index.[20] Calibration plots were generated to assess model bias.

A survival CART model identified subgroups with distinct BCR-free survival, requiring a minimum node size of 10 patients. The median time to BCR or censoring,

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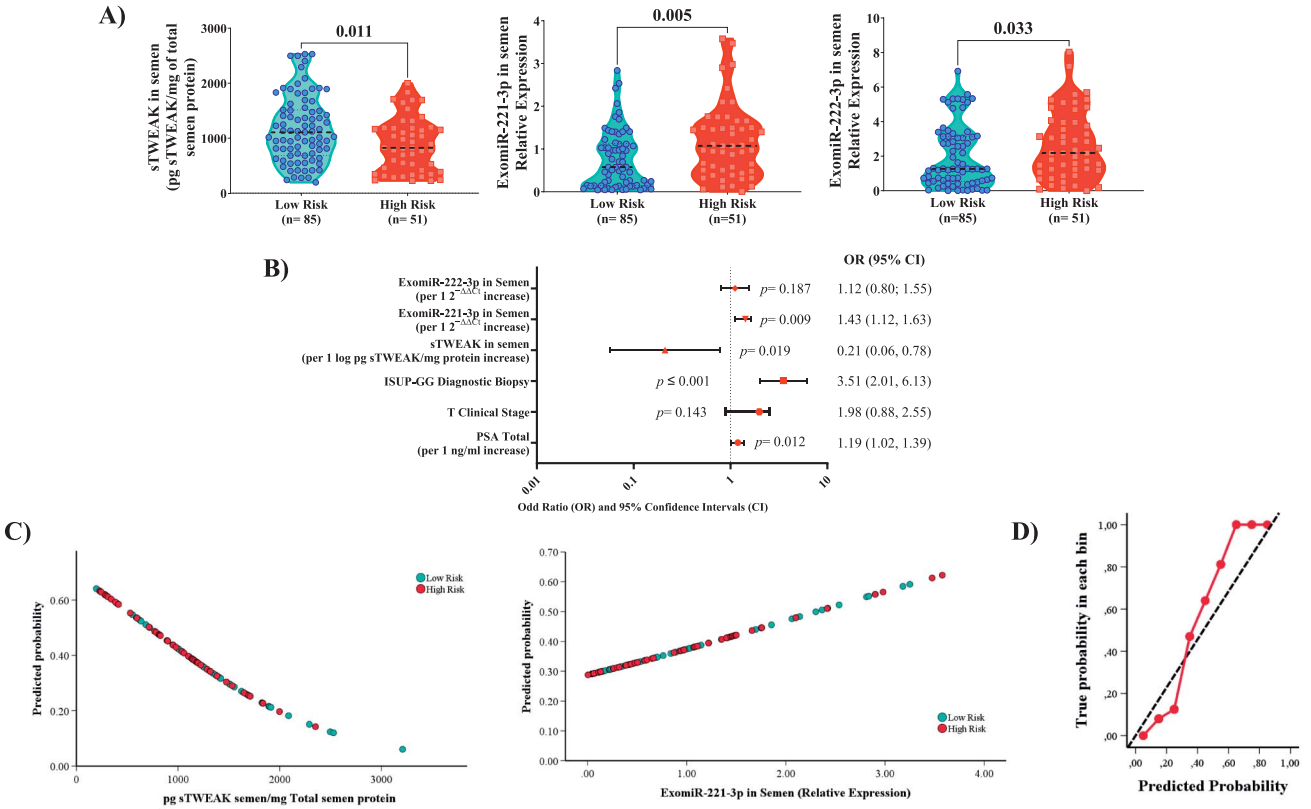


Figure 1. Prognostic performance of the semen biomarkers sTWEAK, exomiRNAs 221-3p, and 222-3p in prostate cancer risk assessment. A, Violin plots representing cytokine sTWEAK levels and relative expression of exomiRNAs 221-3p and 222-3p in semen samples of patients stratified regarding prostate cancer risk assessment: low risk (International Society of Urological Pathology I and II) and high risk (≥III). B, Forest plot from the multivariate logistic regression model with relative expression of exomiR-221-3p and exomiR-222-3p in semen, sTWEAK levels in semen, International Society of Urological Pathology Grade Group (ISUP-GG) diagnostic biopsy, T clinical stage, and PSA levels in serum. OR values for each parameter are represented in different red shapes, along with 95% CIs. Dotted vertical line: OR greater than 1 indicates high values of the variable are likely to occur in high-risk patients. OR less than 1 indicates high values of the variable are likely to occur in low-risk patients. C, Representation of the continuous values sTWEAK and exomiR-221 relative to the predicted probabilities using binary logistic regression. D, Calibration plot assessing the alignment between predicted probabilities and observed outcomes. The x-axis shows predicted probabilities, and the y-axis shows observed probabilities. The dashed line represents ideal calibration, while the red line connects points grouped by probability bins, illustrating the model's calibration.

HRs, and Kaplan-Meier curves was reported to estimate recurrence-free survival.[21] Model discrimination was assessed with time-dependent AUC at 1, 2, and 3 years using the timeROC package.

For diagnostic and risk stratification purposes, multivariate logistic regression (MLR) was applied to estimate odds ratios with 95% CIs. Predictor performance was evaluated through ROC curves and AUC values, while the McNemar χ^2 test assessed differences in sensitivity and specificity between models.

Analyses were performed using SPSS v29 and R v 4.4.3. Statistical significance was set at $P < .05$.

RESULTS

Assessing the Prognostic Accuracy of the sTWEAK, exomiR-221-3p, and exomiR-222-3p Panel for PCa Risk Assessment and BCR Evaluation

We assessed the prognostic accuracy of sTWEAK, exomiR-221-3p, and exomiR-222-3p in semen from 136 patients, divided into low-risk (prostatectomy ISUP I-II, n = 85) and high-risk (prostatectomy ISUP ≥ III, n = 51) groups (Table 1). The results showed that high-risk PCa patients had significantly lower sTWEAK levels and higher exomiR-221-3p and exomiR-222-3p levels compared with low-risk patients (Figure 1, A), confirming our previous findings.[22]

MLR analysis identified exomiR-221-3p, sTWEAK, PSA, and ISUP-GG in the diagnostic biopsy as independent predictors of high-risk PCa, whereas exomiR-222-3p was not significantly associated (Figure 1, B). Subsequent binary logistic regression and ROC curve analysis showed that the combined biomarker panel composed by sTWEAK plus exomiR-221-3p had the highest diagnostic accuracy (AUC = 0.937), outperforming traditional biomarkers such as PSA and ISUP-GG in biopsy (AUC = 0.796; Supplemental Table 1a, https://www.jurology.com). Figure 1, C, shows the continuous variables from the best logistic regression model with their predicted probabilities, demonstrating a linear association and confirming the absence of a justified cutoff threshold.

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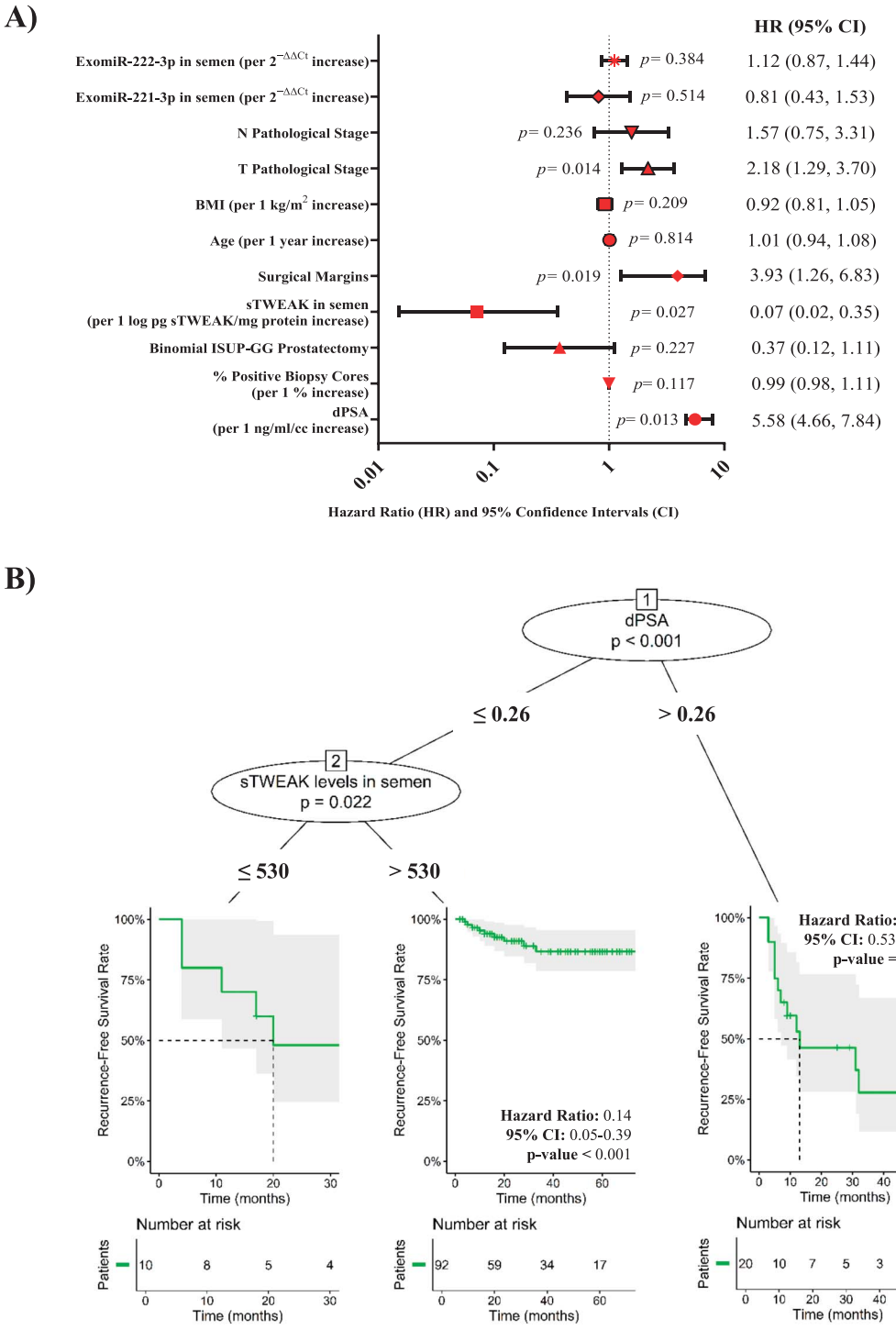


Figure 2. Assessing biochemical recurrence of prostate cancer using the semen biomarkers sTWEAK, exomiR-221-3p, and exomiR-222-3p. A, Forest plot from the multivariable Cox proportional hazards regression model: PSA density (dPSA), percent of positive biopsy cores, binomial International Society of Urological Pathology Gleason Grading (ISUP-GG) prostatectomy, sTWEAK levels in semen, surgical margins, age, BMI, T pathological stage, N pathological stage, and the relative expression of exomiR-221-3p and exomiR-222-3p. The HR and 95% CI are shown. Dotted vertical line: HR greater than 1 indicates high values of the variable are likely related with biochemical recurrence event. HR less than 1 indicates high values of the variable rarely occur in biochemical recurrence event. B, Survival classification and regression tree analysis for biochemical recurrence–free survival based on postoperative dPSA and sTWEAK levels in semen. The first split was determined by dPSA (cutoff: 0.26 ng/mL, $P < .001$). Among patients with dPSA $\leq$ 0.26 ng/mL, further stratification based on sTWEAK levels in semen (cutoff: 530 pg/mL, $P = .022$) revealed distinct prognostic subgroups. Kaplan-Meier curves illustrate recurrence-free survival in each terminal node, with corresponding HR, 95% CIs, and *P* values calculated using the log-rank test. Nodes with $<$ 10 patients were not further split.

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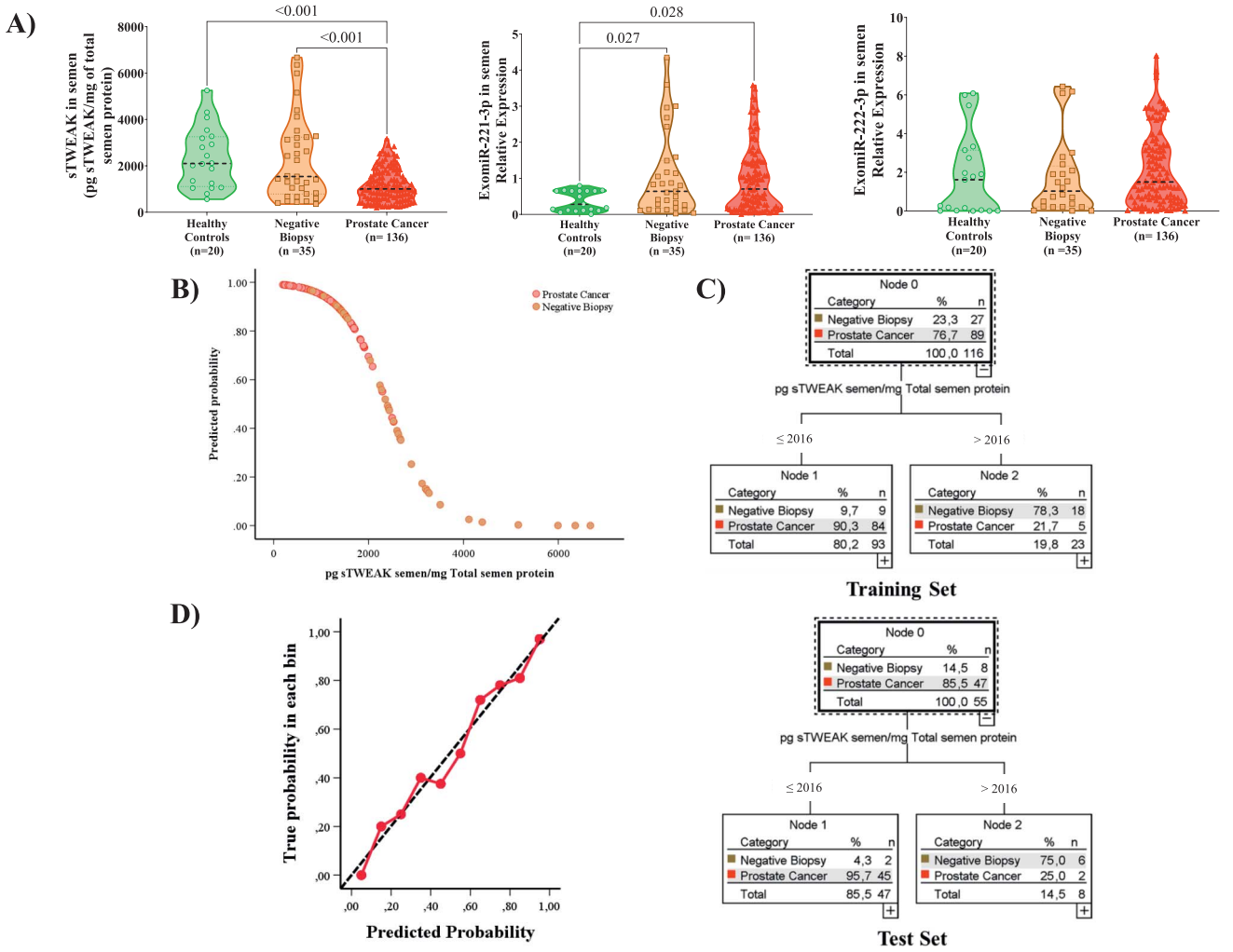


Figure 3. Evaluating the diagnostic potential of sTWEAK and exomiR-221-3p/222-3p biomarkers for prostate cancer. A, Violin plots representing cytokine sTWEAK values and the relative expression of exomiRNAs 221-3p and 222-3p in semen samples from patients with prostate cancer, patients with negative biopsy, and healthy controls group. B, Classification and regression decision tree model for prostate cancer diagnosis (training set and test set). The decision tree was built up with the variable sTWEAK levels in semen (pg sTWEAK/mg of total semen protein). C, Representation of the continuous values of sTWEAK in relation to the predicted probabilities using binary logistic regression. D, Calibration plot assessing the alignment between predicted probabilities and observed outcomes. The x-axis shows predicted probabilities, and the y-axis shows observed probabilities. The dashed line represents ideal calibration, while the red line connects points grouped by probability bins, illustrating the model's calibration.

The calibration plot (Figure 1, D) indicates a good fit for predicted probabilities between 0.0 to 0.3 and 0.8 to 1.0. However, the model overestimates risk in the 0.3 to 0.6 range, potentially leading to misclassification, particularly overestimating patient's risk.

We then assessed the biomarker panel's prognostic value for BCR using Cox regression; the results are shown in a forest plot (Figure 2, A) with HRs and 95% CIs for multiple variables in relation to the risk of BCR over time. Several variables showed statistically significant associations with the outcome ($P < .05$). Notably, T pathological stage (HR = 2.18; 95% CI: 1.29-3.70; $P = .014$), positive surgical margins (HR = 3.93; 95% CI: 1.26-6.83; $P = .019$), sTWEAK levels in semen (HR = 0.07; 95% CI: 0.02-0.35; $P = .027$), and dPSA levels (HR = 5.58; 95% CI: 4.66-7.84; $P = .013$) were independently associated with BCR risk. Schoenfeld residual analysis confirmed that none of the covariates used in the Cox regression model violated the proportional hazards assumption (Supplementary Figure 1, https://www.jurology.com).

The model combining T stage, positive margins, seminal sTWEAK, and dPSA levels showed the highest diagnostic accuracy over time (AUCs: 0.943 at 1 year, 0.880 at 2 years, 0.891 at 3 years; Supplementary Table 1b, https://www.jurology.com). Survival CART analysis (Figure 2, B) revealed dPSA (cutoff: 0.26 ng/mL, $P < .001$) as the primary split. Among patients with dPSA $\leq$ 0.26, those with sTWEAK > 530 pg/mL had a significantly lower recurrence risk (HR = 0.14;

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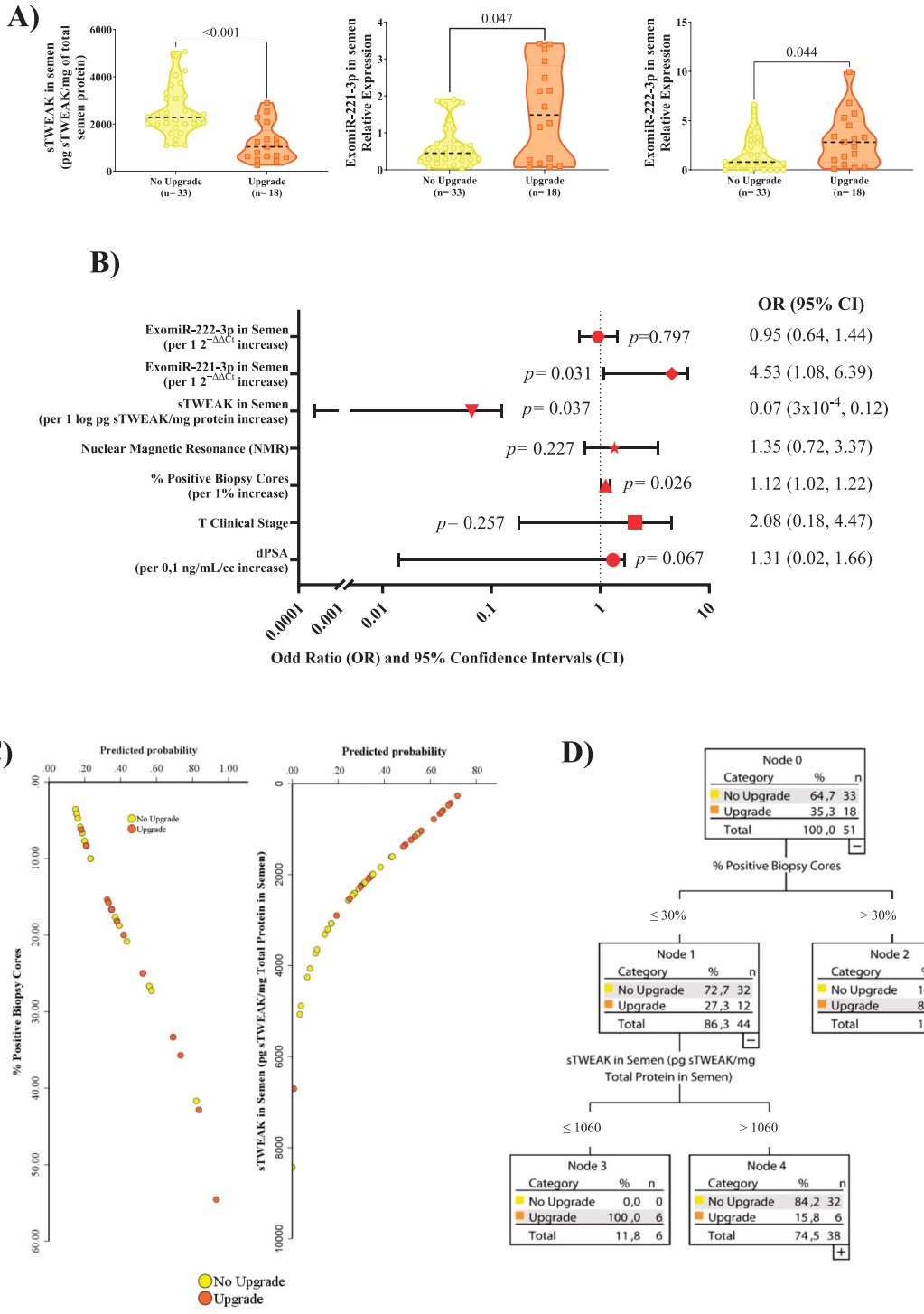


Figure 4. Evaluation of the semen biomarkers sTWEAK, exomiR-221-3p, and exomiR-222-3p for detecting prostate cancer upgrading during active surveillance. A, Violin plots representing cytokine sTWEAK values and the relative expression of exomiRNAs 221-3p and 222-3p in semen samples in a cohort of patients stratified as International Society of Urological Pathology grade I in the diagnostic biopsy and confirmed by a second biopsy, compared with those patients who were upgraded to higher grade in the confirmation biopsy. B, Forest plot from the binary logistic regression model with relative expression of exomiR-221-3p and exomiR-222-3p in semen, sTWEAK levels in semen, NMR, percent of positive biopsy cores, T clinical stage, and PSA density (dPSA) levels. OR values for each parameter are represented in different red shapes, along with 95% CIs. Dotted vertical line: OR greater than 1 indicates that high values of the variable are more likely to occur in upgrading. OR less than 1 indicates that high values of variables that rarely occur in upgrading. C, Representation of the continuous values of sTWEAK and percentage of positive biopsy cores in relation to the predicted probabilities using binary logistic regression. D, Classification and regression decision tree model for upgrading. The decision tree was built with the following parameters: % positive biopsy cores and the levels of sTWEAK in semen (pg sTWEAK/mg of total protein).

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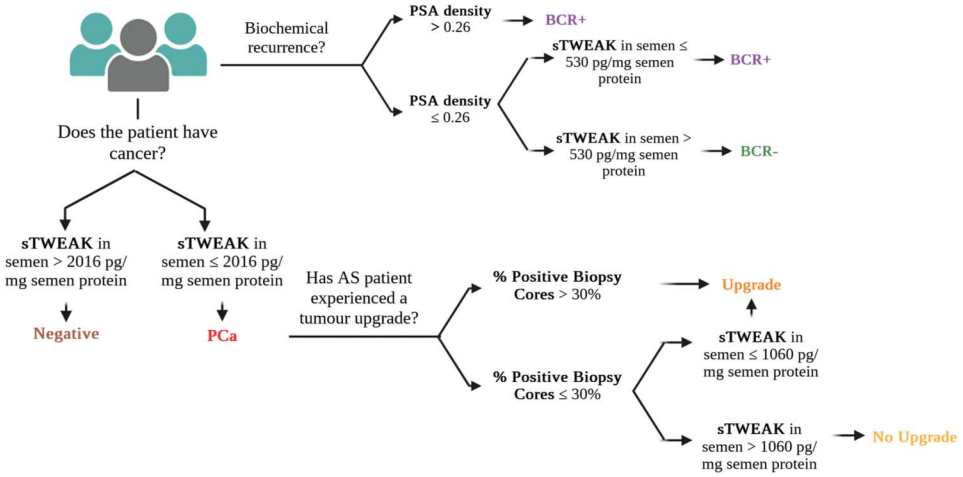


Figure 5. Summary of the use of biomarkers sTWEAK and exomiR-221-3p in prostate cancer (PCa) stratification. This road map shows how to use both biomarkers (sTWEAK and exomiR-221-3p) to help guide PCa management. When semen sTWEAK levels are ≤ 2016 pg/mg, the patient has a high likelihood of having PCa. During active surveillance (AS) follow-up, if the % of positive biopsy cores is ≤ 30%, continue monitoring sTWEAK levels. If sTWEAK drops below ≤ 1060 pg/mg, close monitoring is required as this may indicate tumor progression. For biochemical recurrence (BCR) monitoring: patients with PSA density > 0.26 are likely to relapse, as are those with sTWEAK levels ≤ 530 pg/mg and PSA density ≤ 0.26.

95% CI: 0.05-0.39; $P < .001$), while those with sTWEAK ≤ 530 had poorer BCR-free survival. By contrast, patients with dPSA > 0.26 had similar risk to the low dPSA/low sTWEAK group (HR = 1.41; 95% CI: 0.53-3.76; $P = .49$). These results support using dPSA and sTWEAK jointly for better BCR risk stratification.

Diagnostic Value of the sTWEAK, exomiR-221-3p, and exomiR-222-3p Panel for PCa

We explored the potential of the sTWEAK, exomiR-221-3p, and exomiR-222-3p panel for diagnosing PCa by examining 3 groups: 20 healthy controls, 35 patients with elevated PSA levels (>3.5 ng/mL) but negative biopsy, and 136 patients with confirmed PCa diagnoses based on positive biopsies (Table 2).

Our findings revealed that sTWEAK levels in semen decreased significantly in the PCa group as compared with the healthy controls and negative biopsy groups, with no notable difference between the healthy and negative biopsy groups (Figure 3, A). ExomiR-221-3p expression increased in the negative biopsy and PCa groups compared with the healthy control group, with no significant differences between the negative biopsy and PCa groups. Conversely, no significant differences were observed in exomiR-222-3p levels across the groups (Figure 3, A).

Logistic regression and ROC curve analyses indicated that sTWEAK levels were better at classifying PCa patients, achieving an AUC = 0.897, compared with PSA levels, which had an AUC = 0.598 (Supplemental Table 1c, https://www.jurology.com). Figure 3, B, showed a nonlinear association between continuous values of sTWEAK with predicted probabilities. Thus, CART analysis training set was performed with sTWEAK levels as the independent variable and PCa status (positive vs negative biopsy) as the dependent variable. The analysis revealed that 94.4% of patients with sTWEAK levels ≤ 2016 pg/mg were accurately identified as having PCa (84/89; Figure 3, C). Cross-validation demonstrated the model's robustness, correctly identifying 95.7% (45/47) of the patients in the test set (Figure 3, C, and Supplemental Table 2a, https://www.jurology.com). sTWEAK level measurement in semen reduces PSA overdiagnosis by correctly identifying 68.6% of negative biopsies, while maintaining a high accuracy of 94.9% for cancer detection, compared with PSA, which only identified 5.7% of negative biopsies (Supplemental Figure 2a, https://www.jurology.com). The calibration plot shows good overall alignment with the diagonal, indicating proper calibration. Slight underestimation occurs in the 0.6 to 0.8 range and overestimation in the range of the predicted probability of 0.8 to 1.0 (Figure 3, D).

Utility of sTWEAK, exomiR-221-3p, and exomiR-222-3p Panel for Detecting Upgrading During AS

We assessed the effectiveness of the sTWEAK, exomiR-221-3p, and exomiR-222-3p panel in identifying upgrades in patients under AS. The AS cohort was divided into 2 groups based on confirmation biopsy results: those with ISUP > I (n = 18) and those with ISUP = I (n = 33).

Univariate analysis revealed significant decrease in sTWEAK and significant increase in exomiR-221-3p and exomiR-222-3p levels between compared groups (Figure 4, A).

An MLR analysis was performed using exomiR-221-3p, exomiR-222-3p, sTWEAK in semen, dPSA, the

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percent of positive biopsy cores, NMR, and T clinical stage. Lower sTWEAK levels, higher exomiR-221-3p expression, and a greater percent of positive cores were independently associated with biopsy upgrading (Figure 4, B).

The optimal biomarker combination for detecting upgrading was identified by logistic regression and ROC analysis using sTWEAK in semen, exomiR-221-3p, percent of positive biopsy cores, dPSA, and a clinical panel (dPSA + percent of positive cores + NMR). The exomiR-221-3p + percent of positive biopsy core panel had the highest AUC (0.848), followed by sTWEAK + percent of positive biopsy cores (AUC = 0.824; Supplemental Table 1d, https://www.jurology.com). No significant differences in the ROC curve area, specificity, and sensitivity ($P = 1$) were found between both panels (Supplemental Figure 3a, https://www.jurology.com). Figure 4, C, shows a nonlinear association between both continuous variables and their predicted probabilities. Thus, a CART model was developed using sTWEAK levels and the percent of positive biopsy cores to predict biopsy upgrading. The model showed that upgrades could be predicted with 66.7% accuracy when the percent of positive biopsy cores was $\leq$ 30% and sTWEAK levels were $\leq$ 1060 pg/mg (Figure 4, D). Similarly, a CART tree using the percent of positive biopsy cores and exomiR-221-3p also correctly classified 66.7% of upgrading cases (12/18; Supplemental Figure 3b and Supplemental Table 2b, https://www.jurology.com).

The new panel improves the prediction of upgrade by correctly identifying 66.7% of patients, compared with just 33.3% with the percentage of affected cylinders alone, while maintaining the same accuracy for predicting no upgrade (96.8%; Supplemental Figure 2b, https://www.jurology.com).

DISCUSSION

Liquid biopsies are essential for PCa, providing a noninvasive and accurate method for detecting aggressive tumors.[23] Urine-based biomarkers such as PCA3, MiPS, and ExoDx aid biopsy decisions after elevated PSA. Advances in microRNA panels, including an 18-gene urine assay, show high sensitivity and specificity for PCa detection.[24] However, further validation is needed.

Serum miRNA panels, such as miR-141, miR-100, and miR-1290,[25] show high diagnostic accuracy, although concerns about reproducibility and comparison with PSA persist. While 4Kscore and Prostate Health Index are established for predicting PCa risk, their integration with other diagnostics, such as mpNMR, is debated.[26] Semen, although underexplored, may offer valuable insights into PCa.

We previously proposed a semen-based biomarker panel (exomiR-221-3p, exomiR-222-3p, and sTWEAK) that correctly classified 87.5% of aggressive PCa cases (AUC = 0.857),[22] outperforming the classical PSA test.[27] In a cohort of 136 patients, sTWEAK and exomiR-221-3p emerged as the most significant markers, forming a refined panel with 88% accuracy (AUC = 0.937). The linear association between biomarkers and predicted probabilities does not support the use of an arbitrary cutoff, emphasizing the need for a continuous approach to risk stratification. Future studies could refine thresholds based on clinical outcomes. Unlike recent urine tests analyzing 18 genes,[24] our panel evaluates only 2 biomarkers, offering potential cost advantages.

Our results identify dPSA and seminal sTWEAK levels as independent predictors of BRC in PCa, and it demonstrated high diagnostic accuracy over time. Survival CART analysis revealed distinct prognostic subgroups, supporting the potential role of dPSA and sTWEAK in enhancing postoperative risk stratification and guiding personalized decision-making in PCa management.

In addition, this study explored the usefulness of the 3 biomarkers for PCa diagnosis, aiming to improve specificity and accuracy beyond PSA tests and biopsies. sTWEAK levels (AUC = 0.897) outperformed the PSA test (AUC = 0.598) and reported semen-based miRNA panels. Specifically, sTWEAK levels measurement in semen outperforms the diagnostic potential of other semen-isolated panels such as the miRNA-200c and miR-125b panel (AUC = 0.869),[28] as well as other miRNA and PSA combined panels such as the miR-142-3p + miR-142-5p + miR-223-3p panel (AUC = 0.821) and the miR-342-3p + miR-374b-5p panel (AUC = 0.891).[29]

The selected biomarkers' ability to classify AS-eligible patients was assessed to reduce overtreatment and undertreatment risks. While AS criteria and monitoring vary, effective biomarkers could standardize care by reducing biopsy frequency and complications. The results indicate that men with $\leq$ 30% positive biopsy cores and sTWEAK is above 1060 pg/mL, can continue with AS. The proposed panel predicts tumor upgrading with 66.7%, outperforming the clinical panel (positive biopsy cores).

Figure 5 presents a step-by-step road map for assessing PCa risk. Initially, if semen sTWEAK levels are $\leq$ 2016 pg/mg, the patient has a high likelihood of having PCa. During AS, if biopsy cores are $\leq$ 30%, monitor sTWEAK levels closely; a drop below $\leq$ 1060 pg/mg could suggest tumor progression. For BCR, patients with sTWEAK $\leq$ 530 pg/mg or with sTWEAK $>$ 530 pg/mg and dPSA$>$ 0.26 are at higher risk of BCR.

Despite the potential of these biomarkers, the study has limitations: (1) the biomarker thresholds reported here should be viewed as exploratory and will require refinement and validation in larger, multicentered studies to ensure their generalizability across diverse

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clinical settings; (2) future research should address semen quality variability across age groups and health conditions to refine biomarker accuracy; and (3) optimal integration with other diagnostic methods, such as NMR, is necessary.

CONCLUSIONS

The integration of seminal biomarkers, such as sTWEAK and exomiR-221-3p, with clinical parameters shows potential for enhancing PCa diagnosis, predicting BCR, and monitoring disease progression during AS.

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REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2018;68(6):394-424. doi:10.3322/caac.21492

2. Postema AW, Scheltema MJV, Mannaerts CK, et al. The prostate cancer detection rates of CEUS-targeted versus MRI-targeted versus systematic TRUS-guided biopsies in biopsy-naïve men: a prospective, comparative clinical trial using the same patients. *BMC Urol.* 2017;17(1):27-28. doi:10.1186/s12894-017-0213-7

3. Vargas HA, Hötker AM, Goldman DA, et al. Updated Prostate Imaging Reporting and Data System (PIRADS v2) recommendations for the detection of clinically significant prostate cancer using multiparametric MRI: critical evaluation using whole-mount pathology as standard of reference. *Eur Radiol.* 2016;26(6):1606-1612. doi:10.1007/s00330-015-4015-6

4. Willemse PPM, Davis NF, Grivas N, et al. Systematic review of active surveillance for clinically localised prostate cancer to develop recommendations regarding inclusion of intermediate-risk disease, biopsy characteristics at inclusion and monitoring, and surveillance repeat biopsy strategy. *Eur Urol.* 2022;81(4):337-346. doi:10.1016/j.eururo.2021.12.007

5. Kinsella N, Stattin P, Cahill D, et al. Factors influencing men's choice of and adherence to active surveillance for low-risk prostate cancer: a mixed-method systematic review. *Eur Urol.* 2018;74(3):261-280. doi:10.1016/j.eururo.2018.02.026

6. Pattenden TA, Samaranayke D, Morton A, et al. Modern active surveillance in prostate cancer: a narrative review. *Clin Genitourin Cancer.* 2023;21(1):115-123. doi:10.1016/j.clgc.2022.09.003

7. Manceau C, Fromont G, Beauval JB, et al. Biomarker in active surveillance for prostate cancer: a systematic review. *Cancers (Basel).* 2021;13(17):4251. doi:10.3390/cancers13174251

8. Ahmed ME, Todenhöfer T, Karnes RJ, Seiler R. Prognostic role of RNA expression molecular biomarkers in prostate and bladder cancers. *Eur Urol Focus.* 2022;8(3):663-666. doi:10.1016/j.euf.2022.06.009

9. Kuhl V, Clegg W, Meek S, et al. Development and validation of a cell cycle progression signature for decentralized testing of men with prostate cancer. *Biomark Med.* 2022;16(6):449-459. doi:10.2217/bmm-2021-0479

10. Farha MW, Salami SS. Biomarkers for prostate cancer detection and risk stratification. *Ther Adv Urol.* 2022;14:17562872221103988. doi:10.1177/17562872221103988

11. Di Meo A, Bartlett J, Cheng Y, Pasic MD, Yousef GM. Liquid biopsy: a step forward towards precision medicine in urologic malignancies. *Mol Cancer.* 2017;16(1):80. doi:10.1186/s12943-017-0644-5

12. Zapaøa P, Dybowski B, Poletajew S, Radziszewski P. What can be expected from prostate cancer biomarkers? A clinical perspective. *Urol Int.* 2018;100(1):1-12. doi:10.1159/000479982

13. Blanker MH, Bosch JL, Groeneveld FPMJ, et al. Erectile and ejaculatory dysfunction in a community-based sample of men 50 to 78 years old: prevalence, concern, and relation to sexual activity. *Urology.* 2001;57(4):763-768. doi:10.1016/s0090-4295(00)01091-8

14. Selth LA, Roberts MJ, Chow CWK, et al. Human seminal fluid as a source of prostate cancer-specific microRNA biomarkers. *Endocr Relat Cancer.* 2014;21(4):L17-L21. doi:10.1530/ERC-14-0234

15. Ruiz-Plazas X, Altuna-Coy A, Alves-Santiago M, et al. Liquid biopsy-based exo-oncomirnas can predict prostate cancer aggressiveness. *Cancers (Basel).* 2021;13(2):250. doi:10.3390/cancers13020250

16. Epstein JI, Egevad L, Amin MB, Delahunt B, Srigley JR, Humphrey PA; Grading Committee. The 2014 International Society of Urological Pathology (ISUP) consensus conference on Gleason grading of prostatic carcinoma: definition of grading patterns and proposal for a new grading system. *Am J Surg Pathol.* 2016;40(2):244-252. doi:10.1097/PAS.0000000000000530

17. Sobin LH, Gospodarowicz MK, Wittekind C. *TNM Classification of Malignant Tumours.* Wiley-Blackwell; 2009.

18. Kim EH, Andriole GL. Prostate-specific antigen-based screening: controversy and guidelines. *BMC Med.* 2015;13:61. doi:10.1186/s12916-015-0296-5

19. Ruiz-Plazas X, Rodríguez-Gallego E, Alves M, et al. Biofluid quantification of TWEAK/Fn14 axis in combination with a selected biomarker panel improves assessment of prostate cancer aggressiveness. *J Transl Med.* 2019;17(1):307-313. doi:10.1186/s12967-019-2053-6

20. Park H, Kwon HC. Improved Gini-index algorithm to correct feature-selection bias in text classification. *IEICE Trans Inf Syst.* 2011;E94-D(4):855-865. doi:10.1587/transinf.e94.d.855

21. Bentzen SM, Vogelius IR. Using and understanding survival statistics—or how we learned to stop worrying and love the Kaplan-Meier estimate. *Int J Radiat Oncol Biol Phys.* 2023;115(4):839-846. doi:10.1016/j.ijrobp.2022.11.035

22. Ruiz-Plazas X, Altuna-Coy A, Alves-Santiago M, et al. Liquid biopsy-based exo-oncomiRNAs can predict prostate cancer aggressiveness. *Cancers.* 2021;13(2):250. doi:10.3390/cancers13020250

23. Connal S, Cameron JM, Sala A, et al. Liquid biopsies: the future of cancer early detection. *J Transl Med.* 2023;21(1):118. doi:10.1186/s12967-023-03960-8

24. Tosoian JJ, Zhang Y, Xiao L, et al; EDRN-PCA3 Study Group. Development and validation of an 18-gene urine test for high-grade prostate cancer. *JAMA Oncol.* 2024;10(6):726-736. doi:10.1001/jamaoncol.2024.0455

25. Manoj A, Ahmad MK, Prasad G, Kumar D, Mahdi AA, Kumar M. Screening and validation of novel serum panel of microRNA in stratification of prostate cancer. *Prostate Int.* 2023;11(3):150-158. doi:10.1016/j.prnil.2023.06.002

26. Robinson HS, Lee SS, Barocas DA, Tosoian JJ. Evaluation of blood and urine based biomarkers for detection of clinically-significant

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prostate cancer. *Prostate Cancer Prostatic Dis.* 2025;28(1):45-55. doi:10.1038/s41391-024-00840-0

27. Eyrich NW, Morgan TM, Tosoian JJ. Biomarkers for detection of clinically significant prostate cancer: contemporary clinical data and future directions. *Transl Androl Urol.* 2021;10(7):3091-3103. doi:10.21037/tau-20-1151

28. Roberts MJ, Chow CWK, Schirra HJ, et al. Diagnostic performance of expression of PCA3, hepsin and mir biomarkers inejaculate in combination with serum PSA for the detection of prostate cancer. *Prostate.* 2015;75(5):539-549. doi:10.1002/pros.22942

29. Barceló M, Castells M, Pérez-Riba M, et al. Seminal plasma microRNAs improve diagnosis/prognosis of prostate cancer in men with moderately altered prostate-specific antigen. *Am J Transl Res.* 2020;12(5):2041-2051.

EDITORIAL COMMENT

This study extends the body of work evaluating seminal markers for prostate cancer (PCa) and evaluates a panel of 3 markers measured in semen as a screening, diagnostic, and a prognostic biomarker.[1] The authors previously published on the translational relationship between the cytokine TWEAK and a variety of exosomal microRNAs (exomRs), notably exomRs-221-3p and 222-3p, in PCa.[2] In this study, the team went on to measure TWEAK, exomR-221-3p, and 222-3p in semen as biomarkers for a variety of PCa outcomes.

Using classification and regression tree analysis, seminal TWEAK levels were found to be a marker for several outcomes including presence of cancer, 3-year biochemical recurrence after prostatectomy, and upgrade on active surveillance with reported AUCs of 0.897 (95% CI: 0.837-0.958), 0.812 (95% CI: 0.710-0.914), and 0.824 (95% CI: 0.717-0.978), respectively. Notably, different TWEAK thresholds were used for each outcome and not all included models had a training and test dataset. Perhaps most impressive was the diagnostic performance of TWEAK with a PCa sensitivity of 95.7% and specificity of 75.0% in a held-out test dataset.

Semen remains an intriguing yet perhaps logistically challenging window into PCa biology.[3] Age, sexual function, and cultural perceptions may reduce its feasibility, particularly in older men at risk for PCa. In addition, studies evaluating the performance of seminal biomarkers in comparison with existing tools (urine, blood, and tissue-based biomarkers as well as MRI) for early detection and risk stratification are needed. Nevertheless, the findings of this study suggest the potential of semen as a biomarker source, yet one with practical hurdles that will need to be addressed to facilitate integration into routine clinical practice.

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REFERENCES

1. Alves-Santiago M, Altuna-Coy A, López-Ribelles P, et al. A semen panel signature comprising sTWEAK cytokine levels and expression of exomiR-221-3p and exomiR-222-3p for assessing prostate cancer prognosis, diagnostic utility, and detection of upgrading during active surveillance. *J Urol.* 2026;215(1):30-43. doi:10.1097/JU.0000000000004749

2. Ruiz-Plazas X, Altuna-Coy A, Alves-Santiago M, et al. Liquid biopsy-based exo-oncomiRNAs can predict prostate cancer aggressiveness. *Cancers (Basel).* 2021;13(2):250. doi:10.3390/cancers13020250

3. Roberts MJ, Chow CWK, Schirra HJ, et al. Diagnostic performance of expression of PCA3, hepsin and miR biomarkers inejaculate in combination with serum PSA for the detection of prostate cancer. *Prostate.* 2015;75(5):539-549. doi:10.1002/pros.22942

REPLY BY AUTHORS

We sincerely thank Drs Monda and Salami[1] from the University of Michigan for their thoughtful and encouraging editorial comments on our study.[2] We greatly appreciate their recognition of the promise of seminal biomarkers in prostate cancer detection and prognosis.

Our results demonstrated that a model combining sTWEAK and exomiR-221-3p achieved excellent accuracy for predicting high-risk prostate cancer (AUC = 0.937), while sTWEAK alone distinguished prostate cancer cases with strong diagnostic performance (AUC = 0.897). In patients on active surveillance, a model integrating sTWEAK levels with the percentage of positive biopsy cores predicted biopsy upgrading with an AUC of 0.824. In addition, sTWEAK combined with PSA density showed prognostic value for biochemical recurrence after radical prostatectomy. These findings highlight the versatility of seminal biomarkers across different clinical scenarios—diagnosis, aggressiveness, recurrence, and upgrading during surveillance.

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We agree with the authors' thoughtful remarks regarding the methodologic considerations and the practical challenges of semen collection in clinical settings. These are fair and important points, and we view them as opportunities for future refinement and validation. At the same time, the strong diagnostic and prognostic performance demonstrated in our study supports the translational potential of seminal biomarkers, particularly when integrated with established clinical tools.

We agree that further validation in larger, independent cohorts, as well as head-to-head comparisons with blood-based, urine-based, and imaging-based biomarkers, will be essential to determine their role in clinical practice. Nevertheless, our data support the integration of sTWEAK and exomiRs into multimodal strategies for risk stratification and personalized prostate cancer management.

We thank the authors once again for their constructive insights and for underscoring the potential of seminal biomarkers to advance prostate cancer care.

REFERENCES

1. Monda SM, Salami SS. Editorial comment: A semen panel signature comprising sTWEAK cytokine levels and expression of exomiR-221-3p and exomiR-222-3p for assessing prostate cancer prognosis, diagnostic utility, and detection of upgrading during active surveillance. *J Urol.* 2026;215(1):42. doi:10.1097/JU.0000000000004772

2. Alves-Santiago M, Altuna-Coy A, López-Ribelles P, et al. A semen panel signature comprising sTWEAK cytokine levels and expression of exomiR-221-3p and exomiR-222-3p for assessing prostate cancer prognosis, diagnostic utility, and detection of upgrading during active surveillance. *J Urol.* 2026;215(1):30-43. doi:10.1097/JU.0000000000004749

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