EUROPEAN UROLOGY ONCOLOGY xxx (xxxx) xxx–xxx

available at www.sciencedirect.com
journal homepage: euoncology.europeanurology.com

European Association of Urology

Immunohistochemical Prostate-specific Membrane Antigen (PSMA) Expression Patterns of Primary Prostate Cancer Tissue as a Determining Factor for Prostate Cancer Staging with PSMA Positron Emission Tomography/Computed Tomography

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Article info

Article history:
Received 27 October 2024
Received in Revised form
19 January 2025
Accepted 17 February 2025

Keywords:
Prostate-specific membrane antigen
Positron emission tomography computed tomography
Prostatic neoplasms
Immunohistochemistry
Predictive value of tests

Abstract

Background and objective: In recent studies, prostate-specific membrane antigen (PSMA) positron emission tomography/computed tomography (PET/CT) has shown accuracy in staging patients diagnosed with prostate cancer. Here, we aim to evaluate the correlation between PSMA immunohistochemical characteristics of prostate cancer (PCa)-positive biopsy cores and whole-mount specimens, and test the predictive role of PSMA expression in biopsy samples for staging PSMA PET/CT.

Methods: A total of 104 patients with high- or intermediate-risk PCa who underwent [68Ga]Ga-PSMA-11 PET/CT before radical prostatectomy were prospectively selected between June 2021 and July 2023. The analysis of immunohistochemical PSMA expression was performed using the Immunoreactive Score (IRS). The correlation between biopsy and final specimen was evaluated using Gwet's agreement coefficient for ordinal variables (AC1). Regression models tested the immunohistochemical PSMA expression in biopsy/vesicoprostatic block and the PSMA PET/CT maximum standardized uptake value (SUVmax).

Key findings and limitations: A statistically significant strong correlation was found between PSMA expression in biopsy and vesicoprostatic block (AC1 = 0.8 [confidence interval {CI} 0.7–0.9], $p < 0.01$). According to the multivariable linear regression models, the IRSs of both the PCa-positive biopsy cores and the index lesion were statistically significant predictors of SUVmax ($\beta = 3.3$, CI 1.5–7.5, $p < 0.01$ and $\beta = 4.9$, CI 1.8–13, $p < 0.01$,

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https://doi.org/10.1016/j.euo.2025.02.012

respectively). Limitations include manual interpretation of immunohistochemistry, potential model overfitting, and a short follow-up.
Conclusions and clinical implications: The immunohistochemical analysis of PSMA expression in PCa-positive biopsy cores showed a high correlation with the whole-mount specimen. The degree of PSMA expression is an independent predictor of SUVmax. The assessment of immunohistochemical PSMA expression in a preoperative setting may have implications for determining a more accurate, patient-specific diagnostic pathway.

ADVANCING PRACTICE

What does this study add?
In this study, we evaluated the predictive role of prostate-specific membrane antigen (PSMA) expression in biopsy samples for staging PSMA positron emission tomography/computed tomography in patients diagnosed with prostate cancer. We demonstrated that the immunohistochemical analysis of PSMA expression in biopsy samples can provide data consistent with the characteristics of the prostate tumor itself. The immunohistochemical PSMA expression of the biopsy cores and the index lesion in the final pathology predicted the maximum standardized uptake value. Considering our results, the assessment of PSMA expression in prostate biopsies has great potential to be implemented as an additional element in the preoperative management of patients with prostate cancer.

Clinical Relevance
Does PSMA-PET/CT provide quantitative values for the local staging of prostate cancer ? This remains an open question in an era where PSMA PET/CT is becoming the gold standard for metastatic and nodal staging at prostate cancer diagnosis. In this study, the authors have investigated the correlation of PSMA expression levels (immunoreactice score) with the location of tumor in the prostate, through positive biopsies cores and index lesion at radical prostatectomy specimens. Their positive findings builds upon PSMA immunohistochemistry as a predictive marker for accurate staging, and a surrogate for SUVmax. Associate Editor: Guillaume PLOUSSARD, MD.

Patient Summary
In this report, we evaluated the correlation between prostate-specific membrane antigen (PSMA) immunohistochemical characteristics of prostate cancer–positive biopsy cores and whole-mount specimens. We tested the predictive role of PSMA expression in biopsy samples for staging PSMA positron emission tomography/computed tomography. We found that the immunohistochemical analysis of PSMA expression in biopsy samples can provide data consistent with the characteristics of the prostate tumor itself. The immunohistochemical PSMA expression of the biopsy cores and the index lesion in the final pathology predicted the maximum standardized uptake value.

1. Introduction

Recent studies have demonstrated the superiority of the prostate-specific membrane antigen (PSMA) positron emission tomography/computed tomography (PET/CT) compared to conventional imaging in terms of accuracy in staging patients diagnosed with prostate cancer (PCa) [1–3].

The probability of PSMA PET/CT positivity is influenced by several parameters, and different predictive models have been proposed to select patients who might benefit most from PSMA PET/CT imaging [4–6].

An immunohistochemical analysis of PSMA expression in the final histological specimen has been shown to be a useful tool for interpreting PSMA PET/CT [7–10].

Nevertheless, PSMA expression can be heterogeneous in both primary tumors and distant metastases [11].

Furthermore, 5–10% of primary PCa cases lack significant uptake of PSMA tracers, potentially leading to false-negative results despite prostate-specific antigen (PSA) levels [4,11–14].

Determination of the histopathological and molecular factors underlying the heterogeneity of PSMA expression within and between patients is an unmet need as it can impact the accuracy of PSMA PET/CT.

The use of advanced techniques including immunohistochemistry (IHC) in the preoperative context could guide the selection of the most appropriate diagnostic imaging based on the unique characteristics of each patient [15].

Only one study in the literature compared the PSMA expression on both prostate biopsy cores and pathological specimens with PSMA PET/CT findings [16]. The authors found a positive correlation between immunohistochemical

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features of prostate biopsy cores and the maximum standardized uptake value (SUVmax) at PSMA PET/CT, although not as high as expected [16].

We performed a prospective study to analyze the correlation between immunohistochemical characteristics of PCa in biopsy cores and whole-mount specimens. Moreover, we evaluated the predictive role of immunohistochemical PSMA expression in PCa-positive prostate biopsy specimens in relation to PSMA PET/CT parameters in the primary staging.

2. Patients and methods

2.1. Study population

Consecutive patients diagnosed with high- or intermediate-risk PCa according to the European Association of Urology (EAU) guidelines [17] who underwent [^{68}Ga]Ga-PSMA-11 PET/CT (PSMA PET/CT) for staging proposal were prospectively selected between June 2021 and July 2023 in our tertiary center.

Patients were considered eligible for the study in case of PCa confirmed by a prostate biopsy (performed at our center using a transrectal approach) and recommended with surgical treatment.

The patients underwent either targeted biopsies (TBs) + standard biopsies (SB) or SBs only, based on multiparametric magnetic resonance imaging (mpMRI).

SBs consisted of a 12-core double-sextant template from lateral to medial of the base, mid, and apex.

All patients underwent preoperative staging with PSMA PET/CT within 4 wk before surgery and mpMRI of the prostate.

For each patient, SUVmax related to the prostate was recorded.

The mpMRI scans performed at our center were recorded using the Prostate Imaging Reporting and Data System (PI-RADS) v2.1 score [18]. The mpMRI scans performed elsewhere were reviewed centrally. In the case of positive MRI, we included only patients with a single target lesion identified on mpMRI.

PET/CT scans were performed following current guidelines [19] and reviewed according to the E-PSMA guidelines [5]. An imaging analysis was performed by an expert nuclear medicine physician (M.B.) on a dedicated workstation (Syngo.via, VB30; Siemens Healthineers). A volume of interest (VOI) was placed over the dominant lesion, if clearly visible on PSMA PET/CT. If lesions were not clearly delineated on PET images, the VOI was placed over the tumor region, in correspondence to the lesion localization on histopathology. To quantify [^{68}Ga]Ga-PSMA-11 uptake, SUVmax was measured.

All patients underwent robot-assisted radical prostatectomy and pelvic lymphadenectomy according to the EAU guideline recommendations [17,20].

After surgery, patients were followed up according to the EAU guidelines [17].

In particular, PSA assessment was recommended 40–45 d after surgery and then every 3 mo during the 1st year, together with a urological consultation. In the event of a complete biochemical response (PSA <0.1 ng/ml), PSA measurement was recommended every 6 mo thereafter.

Patients with previous pelvic radiation therapy or hormonal therapy and/or patients who underwent prostate biopsies at other institutions were excluded (n = 75; see Supplementary Fig. 1).

The study adhered to the guidelines of the Declaration of Helsinki and was approved by the local ethical committee (Regional Ethical Committee of Liguria; registration number 343/2019). All patients provided written informed consent for data recording and analysis.

2.2. Immunohistochemical analysis

For each patient, an immunohistochemical analysis was performed for each core corresponding to the respective International Society of Urological Pathology (ISUP) grade group in the prostate biopsy cores. In the case of multiple positive cores with the same ISUP grade group, the one with the highest tumor percentage was selected.

In addition, IHC was performed on the paraffin-embedded blocks corresponding to the index lesion in the final radical prostatectomy specimen, which was evaluated using whole-mount sectioning [21].

The index lesion was defined as the largest prostate adenocarcinoma nodule on the whole-mount specimen [22].

For PSMA antigen immunostaining (clone EP192, 10 μg/ml; Cell Marque), 2-μm thick sections of paraffin-embedded tissue fixed in formalin were used. IHC was performed on the Benchmark ULTRA platform (Ventana Medical Systems, Tucson, AZ, USA) in an automated procedure. Specifically, antigen retrieval was performed using a borate-EDTA buffer (pH 8) at 98°C for a total of 36 min, followed by incubation with the PSMA antibody at room temperature for 32 min. The UltraView DAB IHC Detection Kit was used to develop the reaction. The sections were counterstained with hematoxylin and analyzed subsequently (Fig. 1).

The immunohistochemical analysis was performed by a specialized uropathologist (N.P.), blinded to the PSMA PET/CT findings.

IHC results were reported using the Immunoreactive Score (IRS) [23].

Briefly, the IRS is calculated by multiplying the score for "percentage of positive cells" by the one for "intensity of staining." This results in a score ranging from 0 to 12 (Supplementary Table 1).

The IRS was additionally reported according to the 4-point classification (a 4-point scale ranging from 0 to 3; Supplementary Table 1) proposed by Kaemmerer et al [23].

For the statistical analysis of the immunohistochemical expression variables on biopsy cores, the mean IRS, the mean 4-point IRS score, and the mean percentage of PSMA-positive cells were considered for each patient.

2.3. Outcomes and endpoints

Clinical and demographic data were collected to characterize the sample. The primary endpoint was to evaluate whether the immunohistochemical features detected in prostate biopsy cores reflect PSMA expression consistently in the final whole-mount specimen. Furthermore, the corre-

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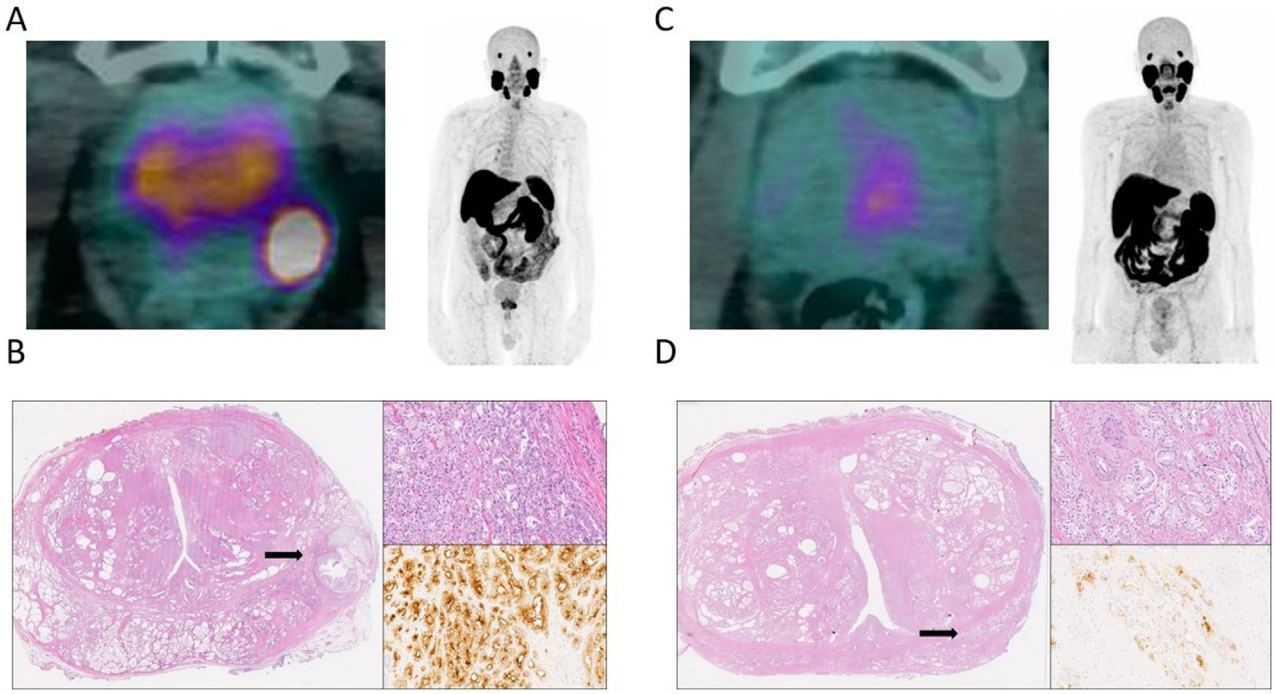

Fig. 1 – Representative examples of high and low PSMA expression in prostate cancer: (A) a PSMA PET/CT scan of prostatic adenocarcinoma with high PSMA expression (left: axial section of fused PET/CT images; right: maximum intensity projection) and (B) corresponding histopathological sample (lesion marked by an arrow in the left image [H&E, 0.15×]; detailed view in the top right [H&E, 20×], with strong PSMA immunostaining in the bottom right image [PSMA, 20×]); (C) a PSMA PET/CT scan of prostatic adenocarcinoma with low PSMA expression (left: axial section of fused PET/CT images; right: maximum intensity projection) and (D) corresponding histopathological sample (lesion marked by an arrow in the left image [H&E, 0.15×]; detailed view in the top right [H&E, 20×], with weak PSMA immunostaining in the bottom right image [PSMA, 20×]). H&E = hematoxylin and eosin; PET/CT = positron emission tomography/computed tomography; PSMA = prostate-specific membrane antigen.

lation between immunohistochemical PSMA expression in the biopsy/vesicoprostatic block and PSMA PET/CT SUVmax was investigated as a secondary endpoint. Finally, short-term oncological follow-up was reported. Specifically, post-operative PSA persistence (PSA >0.1 ng/ml within 4–8 wk after surgery), rate of PSA biochemical recurrence (BCR; PSA >0.2 ng/ml), and any adjuvant or salvage therapies during follow-up were examined.

2.4. Statistical analysis

Descriptive statistics included frequencies and proportions for categorical variables. The mean, median, and interquartile range (IQR) were reported for continuously coded variables. The *Gwet's agreement coefficient* for ordinal variables (AC1) was used to calculate the degree of agreement between PSMA immunohistochemical expression in prostate biopsy cores (calculated as the mean 4-point IRS for each patient) and the index lesion in the final histology [24]. For this analysis, immune expression was categorized as 4-point IRS <2 and 4-point IRS ≥2.

To evaluate whether immunohistochemical characteristics of biopsy cores play a predictive role for PSMA PET/CT parameters in a preoperative setting, uni- and multivariable linear regression models were used to assess the predictive value of the mean IRS (continuously coded, ranging from 0 to 12) for SUVmax (continuously coded). Preoperative variables, including PSA level (continuously coded) and the highest Gleason score in prostate biopsy (7 vs 8–10), were used as covariates to fit the models [10].

Additional uni- and multivariable linear regression models were developed to test the impact of the IRS of the index lesion in the final pathology (postoperative models) on SUVmax (all continuously coded). Covariates for adjustment included the final Gleason score (7 vs 8–10) and tumor volume (continuously coded) [25].

The same analysis was performed only for the subgroup of patients submitted to TBs + SBs (n = 78; Supplementary Table 2).

Moreover, we performed a supplemental analysis to evaluate the association between the IRS of prostate biopsy and pathological outcomes (upgrading and pN1) after radical prostatectomy. Upgrading was defined as an increase in the ISUP score from biopsy to final pathology (Supplementary Table 3).

The R software for statistical computing and graphics (version 4.1.2) was used for all the analyses. All tests were two sided, with a significance level set at $p < 0.05$.

3. Results

The descriptive characteristics of the sample are reported in Table 1.

In total, 104 patients were selected, of whom 42 (41%) had localized intermediate PCa, 47 (45%) had localized high-risk PCa, and 15 (14%) had locally advanced PCa (Table 1).

The median SUVmax of the dominant lesion was 10 (IQR 6, 16; Table 1).

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The final histopathological examination revealed 37 (35.6%) patients with acinar prostatic adenocarcinoma of ISUP grade group 2, 55 (52.9%) with ISUP grade group 3, and 12 (11.5%) with ISUP grade group 4–5 (Table 2).

PSA persistence was reported in 14 (13.5%) patients, and seven (6.7%) patients experienced BCR at a median follow-up of 14 mo (IQR 8, 18). Overall, 15 (14.4%) patients received salvage radiation therapy (Table 2).

The median IRS assessed in biopsy cores was 6 (IQR 4, 8), with 19 (18.2%) patients having a 4-point IRS of <2 and 85 (81.8%) having a 4-point IRS of ≥2.

The median percentage of PSMA-positive cells in biopsy samples was 82% (IQR 53, 95).

For the index lesion in the final histology, the median IRS was 8 (IQR 4, 9), with 18 (17%) patients having a 4-point IRS of <2 and 86 (83%) patients having a 4-point IRS of ≥2 (Table 3). The median percentage of PSMA-positive cells in the index lesion of the final histology was 85% (IQR 68, 95; Table 3).

The simple percentage agreement between the immunohistochemical expression of PSMA on biopsy cores (calculated as the 4-point mean IRS) and on the index lesion of the final histological analysis was 81.8%, with AC1 = 0.8 (confidence interval [CI] 0.7–0.9), which was statistically significant ($p < 0.01$). Similar results were obtained in the subgroup analysis (simple percentage agreement = 80%, AC1 = 0.7 [CI 0.6–0.9], which was statistically significant [$p < 0.01$]).

According to the univariable linear regression analysis, the mean percentage of PSMA-positive cells in the biopsy samples and the mean IRS in the biopsy samples had a significant effect on SUVmax (β = 0.1, CI 0.1–0.3, $p = 0.01$, and β = 1.3, CI 0.5–2.1, $p < 0.01$, respectively; Table 4).

Similarly, the same positive results were reported for the percentage of PSMA-positive cells and for the IRS of the index lesion in the final pathological specimen (β = 0.2, CI 0.1–0.3, $p < 0.01$, and β = 1.5, CI 0.8–2.3, $p < 0.01$, respectively; Table 4).

Table 1 – Descriptive characteristics of 104 patients who underwent robot-assisted radical prostatectomy (RARP) at a single high-volume center between June 2021 and July 2023

Parameter	RARP (n = 104)
Age at surgery (yr), median (IQR)	67 (63, 71)
BMI (kg/m^2), median (IQR)	25.5 (24, 28)
PSA level (ng/ml), median (IQR)	8 (5.1, 11)
Prostate volume [a] (ml), median (IQR)	45 (32, 57)
Prostate biopsy setting before RARP, *n* (%)	
First biopsy	92 (88)
Second biopsy	12 (12)
Biopsy technique, *n* (%)	
Transrectal fusion [b]	78 (75)
Transrectal standard	26 (25)
Number of biopsy cores, median (IQR)	13 (12, 15)
Number of positive biopsy cores, median (IQR)	6 (4, 8)
Biopsy ISUP grade group, *n* (%)	
2	24 (23.1)
3	61 (58.7)
4	15 (14.4)
5	4 (3.8)
mpMRI PI-RADS, *n* (%)	
2	2 (1.9)
3	14 (13.5)
4	59 (56.7)
5	29 (27.9)
EAU risk groups, *n* (%)	
Localized, intermediate risk	42 (41)
High risk	60 (59)
cT stage based on DRE, *n* (%)	
cT1a-2a	67 (64)
cT2b	18 (17)
cT2c	13 (13)
cT3–4	6 (6)
cN+ based on PSMA PET/CT, *n* (%)	26 (25)
SUVmax of the dominant lesion, median (IQR)	10 (6, 16)
ASA score, *n* (%)	
1	25 (24)
2	70 (67.3)
3	9 (8.7)

ASA = American Society of Anesthesiologists physical status classification system; BMI = body mass index; DRE = digital rectal examination; EAU = European Association of Urology; IQR = interquartile range; ISUP grade = International Society of Urological Pathology 2014 grade (group) system; mpMRI = multiparametric magnetic resonance imaging; PET/CT = positron emission tomography/computed tomography; PI-RADS = Prostate Imaging Reporting and Data System; PSA = prostate-specific antigen; PSMA = prostate-specific membrane antigen; RARP = robot-assisted radical prostatectomy; SUVmax = maximum standardized uptake volume.
[a] Prostate volume assessed with mpMRI.
[b] Transrectal fusion: standard biopsies + target biopsies.

Table 2 – Surgical, pathological, and oncological outcomes of 104 patients who underwent robot-assisted radical prostatectomy (RARP) at a single high-volume center between June 2021 and July 2023

Parameter	RARP (n = 104)
OT (min), median (IQR)	220 (190, 250)
EBL (ml), median (IQR)	150 (100, 200)
LOS (d) median (IQR)	5 (4, 7)
RARP ISUP group, *n* (%)	
2	37 (35.6)
3	55 (52.9)
4–5	12 (11.5)
pT stage, *n* (%)	
pT2	62 (59.6)
pT3a	23 (22.1)
pT3b-T4	19 (18.2)
pN stage, *n* (%)	
pN0	89 (85.6)
pN1	15 (14.4)
SM status, *n* (%)	
R0	
R1	
Prostate cancer volume (cc), median (IQR) [a]	9 (7, 18)
Prostate cancer volume (%), median (IQR) [b]	20 (11, 31)
BCR, *n* (%)	7 (6.7)
PSA persistence [c], *n* (%)	14 (13.5)
Adjuvant RT, *n* (%)	6 (5.8)
Adjuvant HT, *n* (%)	4 (3.8)
Salvage RT, *n* (%)	15 (14.4)
Salvage HT, *n* (%)	8 (7.7)
Follow-up (mo), median (IQR)	14 (9, 18)
Lost to follow-up, *n* (%)	2 (1.9)

BCR = biochemical recurrence; EBL = estimated blood loss; HT = hormonal therapy; IQR = interquartile range; ISUP grade = International Society of Urological Pathology 2014 grade (group) system; LOS = length of stay; OT = operative time; PSA = prostate-specific antigen; RARP = robot-assisted radical prostatectomy; RT = radiation therapy; SM = surgical margin.
[a] Whole-mount specimen.
[b] Whole-mount specimen, with respect to the prostate volume.
[c] PSA persistence: after RARP, PSA > 0.1 ng/ml within 4–8 wk after RARP.

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Table 3 – Immunohistochemical features of PCa-positive prostate biopsies and final pathological specimens of 104 patients who underwent robot-assisted radical prostatectomy (RARP) at a single high-volume center between June 2021 and July 2023

Immunohistochemical parameters	RARP (n = 104)
PCa-positive prostate biopsy cores [a]	
IRS, median (IQR)	6 (4, 10)
4-point IRS, median (IQR)	2 (1, 2)
4-point IRS <2, *n* (%)	19 (18.2)
4-point IRS ≥2, *n* (%)	85 (81.8)
PSMA%pos cells, median (IQR)	82 (53, 95)
Index lesion of the final pathological specimens [b]	
IRS, median (IQR)	8 (4, 9)
4-point IRS, median (IQR)	2 (2, 3)
4-point IRS <2, *n* (%)	18 (17)
4-point IRS ≥2, *n* (%)	86 (83)
PSMA%pos cells, median (IQR)	85 (68, 95)

IQR = interquartile range; IRS = immunoreactive score; PCa = prostate cancer; PSMA%pos = percentage of PSMA-positive cells.
[a] For the statistical analysis using the variables of immunohistochemical expression on biopsy cores, the mean IRS value, the mean 4-point IRS value, and the mean percentage of PSMA-positive cells were considered for each patient.
[b] The index lesion was defined as the largest prostate adenocarcinoma nodule on the whole-mount specimen.

According to the multivariable linear regression models, both the mean IRS of the PCa-positive biopsy cores and the IRS of the index lesion were statistically significant predictors of SUVmax, after adjusting for the highest Gleason score at prostate biopsy in the first model and for tumor volume in the second model (β = 3.3, CI 1.5–7.5, $p < 0.01$ and β = 4.9, CI 1.8–13, $p < 0.01$, respectively; Table 4).

The subgroup analysis confirmed the same results (Supplementary Table 4).

4. Discussion

With the widespread use of PSMA PET/CT for PCa staging [1], it is crucial to investigate the histopathological and molecular determinants of heterogeneity in PSMA expression within and between patients.

Our series is the largest one reported in the literature, attempting to characterize PSMA expression in prostate biopsies and postoperative whole-mount specimens.

Moreover, this is the first study that provides predictive models related to the immunohistochemical PSMA expression and SUVmax.

First, we demonstrated a strong statistically significant correlation between the PSMA expression pattern in biopsy cores and the index lesion of the final histological specimen. The immunohistochemical analysis of biopsy samples can provide data consistent with the characteristics of the prostate tumor itself.

Our findings are confirmed by a previous study investigating the prognostic significance of an immunohistochemical PSMA analysis of biopsy cores [10].

More recently, Droghetti et al [16] analyzed 43 patients and demonstrated a moderately positive correlation between the IHC visual score and visual pattern within TB cores, and final pathology, using Cohen's kappa coefficient (0.39 and 0.38, respectively).

We used the Gwet's agreement coefficient for ordinal variables because it is less sensitive to the prevalence of cat-

Table 4 – Univariable linear regression models evaluating the predictive role of immunohistochemical parameters (of both PCa-positive biopsy cores [B] and index lesion of the whole-mount specimen) for SUVmax, and multivariable linear regression models evaluating the predictive role of immunohistochemical parameters of PCa-positive biopsy cores and the index lesion of the whole-mount specimen for SUVmax

Parameter	β	95% CI	p value
Univariable linear regression model evaluating immunohistochemical parameters of PCa-positive biopsy cores and index lesion [a]			
PSA level	0.2	−0.2, 0.5	0.4
IRS [B]	1.3	0.5, 2.1	**<0.01**
PSMA%pos cells [B]	0.1	0.1, 0.3	**0.01**
IRS [P]	1.5	0.8, 2.3	**<0.01**
PSMA%pos cells [P]	0.2	0.1, 0.3	**<0.01**
Highest GS at PB			
7	Ref.		
8–10	8.1	1.1, 15	**0.02**
GS at final pathology			
7	Ref.		
8–10	12	2.4, 22	**<0.01**
PCa volume at final pathology	0.6	0.1, 1	**0.01**
Multivariable linear regression model evaluating immunohistochemical parameters of PCa-positive biopsy cores			
IRS [B]	3.3	1.5, 7.5	**<0.01**
Highest GS at PB			
7	Ref.		
8–10	4	0.4, 13	0.08
Multivariable linear regression model evaluating immunohistochemical parameters of the index lesion of the whole-mount specimen [b]			
IRS [P]	4.9	1.8, 13	**<0.01**
PCa volume at final pathology	1.5	0.9, 2.2	**0.08**

CI = confidence interval; GS = Gleason score; IRS = immunoreactive score; PB = prostate biopsy; PCa = prostate cancer; PSA = prostate specific antigen; PSMA = prostate-specific membrane antigen; PSMA%pos = percentage of PSMA-positive cells; Ref. = reference; SUVmax = maximum standardized uptake value.
[a] For the statistical analysis using the variables of immunohistochemical expression on biopsy cores, the mean IRS value and the mean percentage of PSMA-positive cells were considered for each patient.
[b] The GS at final pathology was not included in the multivariable linear regression model because of the risk of overfitting.

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egories and provides a more stable measure of agreement, particularly in cases where the kappa coefficient may be biased due to unbalanced distributions [26].

Although few studies have explored our topic, these have all expressed an interest in confirming what we have achieved with our analysis [7,9,23,27].

Second, we demonstrated that the mean percentage of PSMA-positive cells and the mean IRS of PCa-positive biopsy cores are predictors of SUVmax. Furthermore, using multivariable regression models, we found that both the IRS of the biopsy cores and the IRS of the index lesion in the final pathology predicted the SUVmax.

These results suggest that PSMA expression in biopsy cores, as well as in the index lesion of the whole-mount specimen, may provide valuable information about the intensity of the PSMA PET/CT signal. This aspect may be particularly important for staging proposals and potentially for restaging BCR, as it could guide the preference for alternative methods in patients with low PSMA expression, such as using conventional imaging for primary staging and choline PET/CT in the restaging setting.

Our results are consistent with some previous evidence in the literature.

Rüschoff et al [27] found that the percentage of PSMA-negative cells in radical prostatectomy specimens is significantly related to a lower SUVmax.

Furthermore, some studies have shown that the intensity of PSMA uptake by the primary tumor in prostate biopsy fragments is an independent predictor of poor prognosis [10], as well as the PSMA immunohistochemical expression in the radical prostatectomy specimen [8,28,29].

Hupe et al [10] found that high PSMA expression in both biopsy and final histology was associated with a higher risk of BCR of the disease.

Based on our results and the data available in the literature, we believe that the assessment of PSMA expression in prostate biopsies has great potential to be implemented as an additional element in the preoperative management of patients with PCa.

Some limitations should be mentioned. First, the interpretation of the slides was performed manually by a pathologist instead of using automated systems, which may lead to some interobserver variability.

As this is an innovative and relatively unexplored topic in literature, the methodology may also be criticized.

We selected patients submitted both to TBs + SBs and to SBs only. The aim was to evaluate the overall relationship between biopsy-derived PSMA expression and PET imaging, taking into account the multifocal nature of PCa and its potential to influence PSMA uptake preoperatively. Given that approximately 30% of clinically significant PCa cases may lie outside of TB samples [30], we chose to include only patients in whom a systematic biopsy was performed (with or without TBs). This decision should ensure that our results capture a broader spectrum of PSMA expression in the preoperative setting.

In the postoperative setting, we concentrated on the index lesion, defined as the largest tumor focus, based on the pragmatic assumption that it contributes most significantly to PSMA immunoreactivity.

The novelty of the approach and the limited number of precedents in this field could raise questions about the reproducibility and generalizability of the results.

Furthermore, we chose to include only patients with a single target lesion identified on mpMRI. Extension of the analysis to patients with multiple target lesions could lead to additional complexity, especially when considering the multifocal nature of PCa and its contribution to PSMA uptake. Inclusion of patients with multiple target lesions represents a useful direction for future research that builds on the foundation laid by the present study.

Although this is the largest available case series with these specific endpoints, the sample size remains limited, and the predictive regression models lack robustness and may be prone to overfitting. Future research will evaluate the potential impact of PSMA expression on the risk of pathological outcomes including pN1 and upgrading after radical prostatectomy, as well as oncological outcomes.

Finally, the follow-up is too short to test the association between PSMA immunoexpression and oncological outcomes. A strength of the study is its prospective design.

5. Conclusions

The immunohistochemical analysis of PSMA expression in PCa-positive biopsy cores showed a high correlation with PSMA expression in the index lesion of the whole-mount specimen. The degree of PSMA expression is an independent predictor of SUVmax. The assessment of immunohistochemical PSMA expression in a preoperative setting may have significant implications for determining a more accurate, patient-specific diagnostic pathway.

***Author contributions*:** Francesca Ambrosini had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Ambrosini, Suardi, Piol, Bauckneht, Mantica.
Acquisition of data: Ambrosini, Piol, Drocchi, Col, Martiriggiano, Vecchio, Paola, Sofia, Celesti, Giasotto.
Analysis and interpretation of data: Ambrosini, Bauckneht, Mantica.
Drafting of the manuscript: Ambrosini, Bauckneht, Mantica.
Critical revision of the manuscript for important intellectual content: Mantica, Terrone.
Statistical analysis: Ambrosini.
Obtaining funding: Mantica, Piol, Bauckneht.
Administrative, technical, or material support: Terrone, Piol, Bauckneht.
Supervision: Terrone, Barra, Giasotto, Fornarini, Sambuceti, Borghesi.
Other: None.

Financial disclosures: Francesca Ambrosini certifies that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or

Please cite this article as: F. Ambrosini, N. Piol, M. Bauckneht et al., Immunohistochemical Prostate-specific Membrane Antigen (PSMA) Expression Patterns of Primary Prostate Cancer Tissue as a Determining Factor for Prostate Cancer Staging with PSMA Positron Emission Tomography/Computed Tomography, Eur Urol Oncol (2025), https://doi.org/10.1016/j.euo.2025.02.012

patents filed, received, or pending), are the following: Matteo Bauckneht reports personal fees from AAA and GE Healthcare outside the submitted work. No other potential conflict of interest relevant to this article was reported.

***Funding/Support and role of the sponsor*:** We extend our sincere gratitude to the Ministry of Health for its financial support through the 5x1000 contribution (C8 44A). This work was supported by the Italian Ministry of Health (research project no. 5M-2019-23680287).

***Data sharing statement*:** The raw data supporting the conclusions are available upon request. The data will be provided to researchers who meet the criteria for access to confidential information and agree to the terms of use.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.euo.2025.02.012.

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