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Detection efficacy of hybrid ^{68}Ga-PSMA ligand PET/CT in prostate cancer patients with biochemical recurrence after primary radiation therapy defined by Phoenix criteria

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Short running title: PSMA-PET in biochemical relapse after RT

ABSTRACT

The aim of this retrospective study was to evaluate the detection rate of Glu-NH-CO-NH-Lys-(Ahx)-[^{68}Ga(HBED-CC)] (^{68}Ga-PSMA ligand) positron emission tomography/computed tomography (PET/CT) in patients with biochemical recurrent prostate cancer (PC) defined by Phoenix criteria after external beam radiotherapy (EBRT) or brachytherapy as primary treatment.

Methods

118 patients were finally eligible for this retrospective analysis with a median prostate-specific antigen (PSA) of 6.4 ng/mL (range: 2.2-158.4 ng/mL, interquartile range: 4.2-10.2 ng/mL). 77 and 41 patients had been treated by EBRT or brachytherapy, respectively. Of the 118 patients, 45 were receiving androgen deprivation therapy (ADT) within at least 6 months prior to the PET/CT. The detection rates were stratified by PSA. The influence of primary Gleason score (GS) and ADT was assessed. Relationships between standardized uptake values (SUV) and clinical as well as pathological features in patients with positive findings were analyzed using univariate and multivariable linear regression models.

Results

90.7% (107/118) patients showed pathological findings indicative for tumor recurrence in ^{68}Ga-PSMA ligand PET/CT. The detection rates were 81.8% (36/44), 95.3% (41/43) and 96.8% (30/31) for PSA of 2 to <5, 5 to <10 and ≥10 ng/mL, respectively (p=0.0377). ^{68}Ga-PSMA ligand PET/CT indicated local recurrence in 68/107 patients (63.5%), only distant lesions in 64/107 patients (59.8%) and local recurrence as well as distant lesions in 25/107 patients (23.4%). The detection rate was significantly higher in patients with ADT (97.7%) vs. without ADT (86.3%, p=0.0381), but independent from primary GS ≥8 (92.0%) vs. ≤7 (90.2%, p=0.6346). SUV_{max} and SUV_{mean} were significantly associated with PSA and ADT (p=0.018 and 0.004 for SUV_{max}, respectively; p=0.025 and 0.007 for SUV_{mean}, respectively) .

Conclusion

^{68}Ga-PSMA ligand PET/CT demonstrates high detection rates in patients with biochemical recurrence of PC after primary radiation therapy. The detection rate was positively associated to increasing PSA as well as concomitant ADT. ^{68}Ga-PSMA ligand PET/CT enables discrimination of local vs. metastatic disease and thus might have a crucial impact on further clinical management. A major limitation of this study is the lack of histopathological proof in the majority of patients.

key words: PSMA ligand, PET/CT, prostate cancer, EBRT, brachytherapy

INTRODUCTION

The most common approaches in the primary treatment of prostate cancer (PC) are radical surgery, external beam radiation therapy (EBRT), brachytherapy (without or in combination with EBRT) and/or androgen deprivation therapy (ADT) (*1*). Following radiation therapy (RT) as the primary treatment of PC, a prostate-specific antigen value (PSA) of 2 ng/mL above the PSA nadir represents biochemical recurrence (BCR) defined by Phoenix criteria which is the current standard of reference for the definition of BCR after primary RT (*2*). Biochemical failure is seen in 10% to 60% of patients after EBRT, depending on pretreatment risk factors and on the radiotherapy technique used (*3*). After brachytherapy, biochemical recurrence after 5 and 10 years was reported to range from 7% to 29% and from 15% to 35%, respectively (*1,4*). Monitoring of PSA is a reliable and cost-effective way to detect disease relapse. However, it cannot differentiate between local, locoregional or systemic recurrence. Imaging modalities such as bone scintigraphy (BS) and computed tomography (CT) exhibit considerable limitations in the setting of PSA <10 ng/mL and may show the site of recurrence only in patients with fast PSA kinetics (PSA velocity >2 ng/mL per year) or higher PSA values (>20 ng/mL) (*5-7*). By contrast, positron emission tomography (PET)/CT with ^{11}C-labelled choline derivatives in patients with BCR at low PSA values after EBRT has proven to be a valuable tool (*8*).

The recent introduction of Glu-NH-CO-NH-Lys-(Ahx)-[^{68}Ga(HBED-CC)] (^{68}Ga-PSMA ligand) as an extracellular PSMA-inhibitor for PET-imaging demonstrated excellent results especially for patients with BCR. It showed markedly improved detection rates in direct head-to-head comparison or compared to data from literature (*9,10*). Most recently, Perera et al presented a review of ^{68}Ga-PSMA ligand PET, demonstrating a pooled detection rate of 76% for ^{68}Ga-PSMA ligand PET/CT (*11*), considerably exceeding the pooled 62% detection rate for ^{11}C-choline PET (*12*). However, most ^{68}Ga-PSMA ligand PET/CT studies have evaluated either an inhomogeneous

patient population, including predominantly patients showing biochemical recurrence after radical prostatectomy and including also a low proportion of patients after EBRT as well as progressive disease, or completely focused on the group of patients after radical prostatectomy (*10,13,14*).

To the best of our knowledge so far no study is published focusing on the clinical performance potential of ^{68}Ga-PSMA ligand PET in recurrent PC patients after primary curative intended RT alone. As localization of relapse in BCR after primary RT is challenging imaging plays a crucial role in further therapy stratification. Therefore, the purpose of our study was to evaluate the detection rate of ^{68}Ga-PSMA ligand PET/CT and to compare it to the results of primary histologic differentiation (Gleason score; GS) and ADT in a large population of patients with BCR according to the Phoenix criteria after primary treatment with EBRT or brachytherapy.

MATERIALS AND METHODS

Patients

183 patients who underwent ^{68}Ga-PSMA ligand PET/CT imaging for recurrent PC after primary RT were extracted from the institutions' database (November 2012 to March 2016). Subsequently patients were excluded in whom BCR according to the Phoenix criteria was not fulfilled. The latter is defined as a PSA rise by 2 ng/mL or more above the nadir PSA after RT. Further exclusion criteria were: salvage radical prostatectomy, transurethral resection of the prostate, cryosurgical ablation of the prostate, high-intensity focused ultrasound, irreversible electroporation, second-line anti-hormonal therapy, chemotherapy and bone targeted therapy with Radium-223 (Fig. 1). In total, 118 patients were enrolled in this retrospective study. Details on patient characteristics are summarized in Table 1.

All patients signed a written informed consent form for the purpose of anonymized evaluation and publication of their data. All reported investigations were performed according to the principles of the Helsinki Declaration and to national regulations. The study was approved by the Ethics Committee of the Technical University Munich (permit 5665/13).

Imaging and Interpretation

A detailed description of ^{68}Ga-PSMA ligand and imaging parameters is available as supplemental material (Supplemental file). All PET/CT images were interpreted by one board-certified nuclear medicine physician and one board-certified radiologist in consensus. All lesions suggestive for recurrent PC were noted and grouped with respect to their localization into local recurrence, lymph node metastases, bone metastases, and other metastases. Imaging findings were validated in 35.5% (38/107) of patients. Further details on the validation criteria are provided as supplemental material (Supplemental file).

In PET, any focal uptake higher than background and not associated with physiologic uptake was judged as tissue suspicious of malignancy. For quantitative assessment, only the highest standardized uptake value (SUV) was noted in each suggestive anatomical field. To calculate SUVs, an isocontour volume of interest including all voxels above 50% of the maximum was created, covering the whole lesion volume, as performed recently (*15*). Within all volumes of interest, mean and maximum SUVs were measured. For CT, any distinct sclerotic lesion not being associated with degenerative changes and any small lung lesion not being related to inflammatory changes or associated with typically subpleural intrapulmonary lymph nodes below the level of the carina (*16*) were considered as positive. Criteria for interpretation of ^{68}Ga-PSMA ligand PET/CT have been recently published (*17*).

Statistical Analysis

The detection rate was plotted against the absolute PSA-value. Two-sided chi-squared tests to evaluate differences between single groups and Mann-Whitney U tests to evaluate differences concerning PSA-values were used. Univariate and multivariable linear regression models were fit to the data to assess the association between SUV (lesion with highest SUV_{max} and SUV_{mean}) and PSA, iPSA, BMI, age, injected activity, acquisition time, GS (GS ≥8 vs. ≤7), ADT (with and without ADT), and the type of radiation therapy. SUVs were logarithmized to account for skewed distributions. A p-value <0.05 was considered significant. Statistical analyses were done with software (GraphPad PRISM 6, MedCalc version 16.8 and IBM SPSS Statistics version 23).

RESULTS

Detection Efficacy

^{68}Ga-PSMA ligand PET/CT showed pathologic findings suggestive for recurrent PC in 107/118 (90.7%) patients. With respect to the PSA-value, the detection efficacy was 81.8% (36/44) for a PSA of 2 to <5 ng/mL, 95.3% (41/43) for a PSA of 5 to <10 ng/mL, and 96.8% (30/31) for a PSA of ≥10 ng/mL (Fig. 2). The detection rates were significantly different according to the 3 different PSA ranges (p=0.0377). Figure 3 is demonstrating the number and percentage of all patients with findings suspicious for recurrent PC separated by different locations. PSA was significantly higher in patients with positive ^{68}Ga-PSMA ligand PET/CT findings than in patients with negative results (p=0.0152; Table 2).

Effect of ADT, GS and Type of Radiation Therapy

Detection efficacy was significantly higher in patients with ADT compared to patients without ADT (p=0.0381; Fig. 2). Suspicious lesions were detected in 97.7% (44/45) of patients with ADT and 86.3% (63/73) of patients without ADT. PSA-values between both patient groups were not significantly different (p=0.087; Table 2).

Considering the histopathologic differentiation of the primary PC, ^{68}Ga-PSMA ligand PET/CT showed positive findings in 90.2% (54/61) of patients with a GS ≤ 7 and 92.0% (23/25) of patients with a GS ≥ 8 (p=0.635; Fig. 2). Again, PSA-values between both patient groups were not significantly different (p=0.056; Table 2).

There was a significantly higher detection rate with respect to the type of primary RT which was 97.4% (75/77) in patients after EBRT and 78.0% (32/41) in patients after brachytherapy (p=0.0006; Fig. 2). PSA-values in these patient groups did not differ significantly (p=0.340; Table 2). However, a significantly higher portion of the patients with EBRT had ADT compared to those with brachytherapy (49.4% vs. 17.1%; p=0.0006). Regarding the type of RT, the detection rates did not differ significantly between patients with and without ADT in this subgroup analysis (Supplemental Fig. 1).

Influence of Clinical and Pathological Features on SUVs

The univariate linear regression analyses showed a significant correlation between PSA-values, GS, ADT and SUVs (all p=<0.04; Supplemental Table 1). In a first multivariable linear regression analyses containing PSA, iPSA, GS, ADT, type of radiation therapy, acquisition time, injected activity, age and BMI, the overall model was significantly superior to a null model without covariates (p=0.024 and 0.030 with respect to SUV_{max} and SUV_{mean} as dependent variables, respectively), but no variable was significantly independently associated with SUV (the analysis was limited to n=67 due to missing data for GS and iPSA; Supplemental Table 2). A second

multivariable linear regression analysis (excluding GS and iPSA data to include more patients; n=107) revealed a significant association of PSA and ADT with SUVs (Table 3).

Histopathology and Follow-up

In 6 patients, ^{68}Ga-PSMA ligand PET/CT positive local recurrence or metastases were histologically confirmed (Fig. 4). In 29 patients, follow-up/imaging (PET/CT, PET/MRI, BS, CT) undisputable proved that the positive findings were metastases or local recurrence of PC. In another 3 patients, RT or chemotherapy followed by a substantial decrease in PSA and/or decreasing PSMA uptake of suspicious findings in a consecutive ^{68}Ga-PSMA ligand PET/CT scan proved the malignant nature of PSMA-positive lesions (Supplemental Fig. 2).

DISCUSSION

To the best of our knowledge, this is the first study investigating the detection efficacy of ^{68}Ga-PSMA ligand PET/CT in a collective of patients with BCR according to the Phoenix criteria after curative intended RT. An overall detection rate of 90.7% indicates that ^{68}Ga-PSMA ligand PET/CT is highly effective in this pre-selected patient group. A significantly higher detection efficacy (at relatively identical PSA-values) as well as a significantly higher SUV for patients with vs. without ADT indicates no need for withdrawal of hormonal treatment as discussed for choline-labelled derivates and highlights the potential of possible improved targeting.

BCR after primary curative intended RT is relatively frequent and ranges from 10% to 60% and 7% to 35% in EBRT and brachytherapy, respectively (*1,3,4*). The aim in these patients is twofold: (1) determine the presence or absence of recurrent disease, (2) determine its exact location(s), as the disease can be local (25%-30% of cases), systemic (20%-25% of cases), or both (45%-55% of

cases) (*18*). Due to the strong limitations of conventional imaging techniques (CT, MRI and bone scintigraphy) in detecting the site or sites of relapse, none of the main international guidelines recommend these imaging procedures for patients with biochemical failure post RT, unless the PSA values are markedly elevated (e.g. PSA > 10 ng/mL) or patients are symptomatic (e.g. pain, fracture, etc.) (*7*). According to our results, ^{68}Ga-PSMA ligand PET/CT may offer the possibility in detecting recurrent PC at a clearly earlier timepoint with the necessary accuracy, that is crucial for further disease management. As an important finding, ^{68}Ga-PSMA ligand PET/CT showed positive findings outside the prostate in 59.8% of patients. Comparable results (i.e. 62.6%) are reported by Ceci et al for ^{11}C-Choline PET/CT (*8*). Unifocal or multifocal local recurrence by means of ^{68}Ga-PSMA ligand PET/CT was present in 63.5% of patients, which is similar to the findings demonstrated by Ceci et al and Breeuwsma et al (62.6% and 71.9%, respectively) (*8,19*). Notably, the detection or exclusion of local recurrence after primary RT and the finding of metastatic disease not amenable for surgical resection are crucial in view of a potential radical salvage prostatectomy in carefully selected patients (PSA <10 ng/mL, PSA doubling time >12 months, low dose brachytherapy, GS <7; according to the European Association of Urology guidelines (*7*)). In addition, precise localization of a limited number of systemic lesions can further advance the increasingly popular concept of treating oligometastatic disease by stereotactic radiation therapy.

To date, transrectal ultrasonography guided biopsy is the current reference standard for the detection of local recurrence in patients with BCR after primary RT. However, it is invasive and may fail to depict some tumors because only a small fraction of the prostate gland is sampled. ^{68}Ga-PSMA ligand PET as a noninvasive promising alternative enabling the assessment of the entire gland could be preferable, which has already shown promising results for primary PC in combination with MRI (*20*). Furthermore, with regard to the high detection rates of ^{68}Ga-PSMA

ligand PET/CT within the prostate and exact localization of extraprostatic disease (frequently lymph node and bone metastases and even uncommon metastatic manifestations; see example in Fig. 5), more personalized and tailored therapy approaches may be achieved. In particular, the detection of local recurrence together with pelvic lymph node metastases (overall 14 cases in our study) may modify the surgical regimen of intended salvage prostatectomy by adding and guiding lymph node dissection, which has been recently shown (*15*). Further studies are warranted to evaluate the role of ^{68}Ga-PSMA ligand PET/CT in the therapeutic management of recurrent PC post RT.

In parallel to other PET-tracers and reports, our data show an increase in detection rate of ^{68}Ga-PSMA ligand PET/CT with rising PSA values (*8,21*). To our knowledge, only three prior reports, involving 46, 70 and 140 patients, respectively, have investigated the value of ^{11}C- or ^{18}F-labelled choline PET/CT imaging in detecting recurrent prostate cancer after EBRT or brachytherapy (*8,19,21*). The detection efficacy of 90.7% for ^{68}Ga-PSMA ligand PET/CT in our patient cohort is slightly higher than compared to fore-mentioned studies ranging between 80.4% to 87.8%. However, with respect to the results of Breeuwsma et al (*19*), the median PSA in our patient population was lower (median PSA: 6.4 ng/mL [range: 2.2-158.4 ng/mL; interquartile range: 4.2-10.2 ng/mL] in our cohort vs. 10.7 ng/mL [range: 0.6-54.7 ng/mL; interquartile range not reported], respectively), indicating a less advanced disease stage in direct comparison and clearly emphasizing the strength of ^{68}Ga-PSMA ligand PET/CT in potentially detecting recurrent PC at an earlier timepoint of BCR. By contrast, the other two studies by Ceci et al (*8*) and Chondrogiannis et al (*21*) had lower PSA values with regard to median PSA or PSA range (median PSA: 5 ng/mL and range: 2-60 ng/mL in the study of Ceci et al and range: 1.1-49.4 ng/mL [median PSA not reported] in the study of Chondrogiannis et al, respectively). Nevertheless, our study demonstrates

substantial detection efficacies for ^{68}Ga-PSMA ligand PET/CT after primary RT, which is in the range of previous studies (68-89%) reported for patients with BCR who had been predominantly treated with radical prostatectomy (*10,13,22*).

Our data show higher detection rates and SUVs ($SUV_{max/mean}$ as a potential biomarker of PSMA-expression) in patients with ADT vs. patients without ADT, confirming histological and immunohistological reports stating a higher PSMA expression of PC cells in the setting of ADT (*23,24*). Notably, relatively comparable PSA-values could be observed between these patient groups excluding mere differences due to higher tumor burden. Therefore, it can be concluded that unless PSA-values are not considerably suppressed, an ongoing ADT or a new onset ADT shortly before ^{68}Ga-PSMA ligand PET/CT seem not to relevantly reduce diagnostic capability.

Besides ADT, statistically significant associations between PSA and SUVs were shown according to univariate and multivariable linear regression analyses which could potentially reflect disease activity. For GS, significant correlations to SUVs were detected in the univariate linear regression model which were not present in the multivariable regression analysis. A positive correlation between increasing GS and PSMA expression is in line with preclinical studies (*25,26*). Moreover, in a recently published large clinical retrospective study, ^{68}Ga-PSMA ligand PET/CT demonstrated a significantly higher detection efficacy in the setting of GS ≥8 in patients with relapsing PC (*13*), which was attributed to a higher PSMA expression in higher GS.

Interestingly, our data indicate a significantly higher detection rate in patients with EBRT compared to brachytherapy (97.4% vs. 78.0%; p=0.0006). However, a significantly higher proportion of patients who were treated with EBRT compared to brachytherapy received ADT within 6 months prior to imaging. Thus, no clear statement on the efficacy of ^{68}Ga-PSMA ligand PET/CT for EBRT vs. brachytherapy can be drawn from these data, as ADT represents a

considerable confounding factor according to the results of the multivariable regression analyses in this study.

To date, the Phoenix criteria are the current standard of reference for the definition of BCR after primary RT (*2*). While these criteria are highly specific to identify PC relapse, they lack in sensitivity (*2*), since a prostate gland treated with RT may still harbour relevant disease requiring further treatment without yet fulfilling the Phoenix criteria. In such cases deferred therapy due to application of current Phoenix criteria may result in worse oncological and/or functional outcomes due to local or distant disease progression. Recently, Meeks et al showed persistent PC after primary RT in 45% of patients submitted to radical cystoprostatectomy, which was performed for bladder cancer at a later time point. Besides, PC was found in 37% of patients without evidence of BCR (*27*), suggesting that many persistent or recurrent PC's may not meet the Phoenix criteria for intervention. Thus, promising biomarkers other than PSA like PSMA should be further evaluated to potentially better identify those with viable PC at an earlier time point after RT. Considering the powerful detection efficacy of ^{68}Ga-PSMA ligand PET/CT in our study and the significant association of early salvage treatment at low PSA values with improved biochemical free survival in PC patients after RT (*28*), PSMA imaging may be performed early in the course of recurrent PC, even if the Phoenix criteria are not fulfilled. However, further studies are needed to evaluate the potential role of ^{68}Ga-PSMA ligand PET/CT at lower PSA values post RT, additionally reconsidering the validity of the Phoenix criteria for detecting PC relapse.

Our study has some limitations. Since it was a retrospective single-institution study, our results may not be generalizable, as imaging acquisitions and interpretation expertise vary across institutions. Despite being retrospective in nature, the particular strength of our study consists in

the patient selection strictly including patients with biochemical failure after primary RT as defined by the Phoenix criteria. Next, we did not evaluate the influence of PSA kinetics (velocity and doubling time) on ^{68}Ga-PSMA ligand PET/CT detection rates. We tried to request the series of PSA-values needed for these calculations, but nevertheless comprehensive data were missing in >80% of patients. However, it has been recently shown that ^{68}Ga-PSMA ligand PET/CT detection rates are not substantially influenced by PSA kinetics (*13*). Finally, histopathology in each patient would have been preferable but was not feasible for practical and ethical reasons.

CONCLUSION

^{68}Ga-PSMA ligand PET/CT demonstrates a high (>90%) detection efficacy in patients with BCR after primary RT according to Phoenix criteria. The detection rate is dependent on the PSA-value as well as enhanced by ADT. The higher detection rate in patients under ADT as well as higher SUVs is compatible with PSMA upregulation under ADT and indicates that unless PSA-values are not considerably suppressed, the withdrawal of ADT prior to ^{68}Ga-PSMA ligand PET/CT is not necessary. ADT could possibly enhance the diagnostic potential by means of target upregulation. ^{68}Ga-PSMA ligand PET/CT can have a crucial impact on further clinical management after BCR in RT-treated patients.

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Figure 1. Flow chart of patient selection. *Transurethral resection of the prostate, **High intensity focal ultrasound and #Irreversible electroporation.

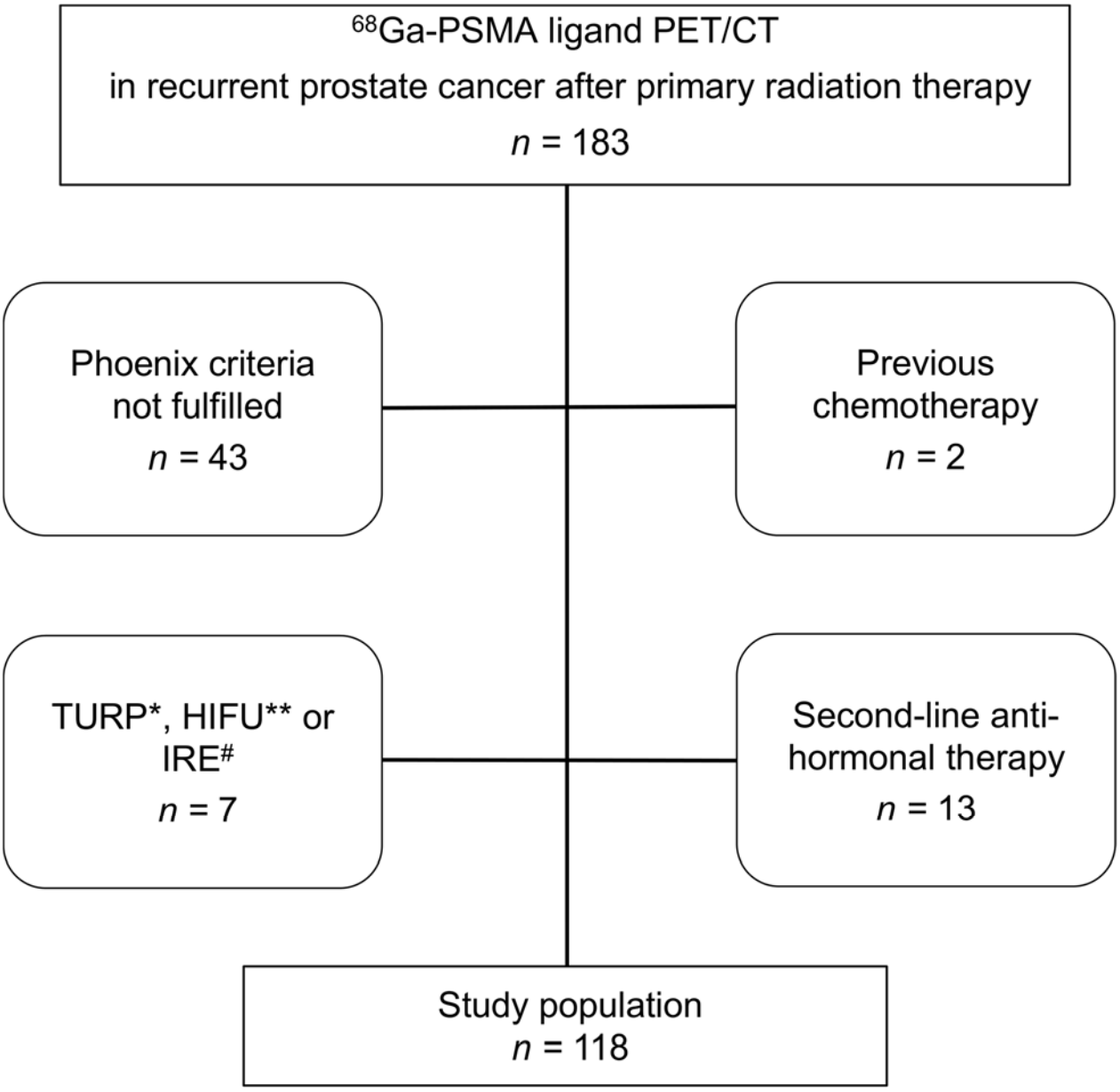


Figure 2. Detection efficacy of ^{68}Ga-PSMA ligand PET/CT in relation to androgen deprivation therapy (A), PSA (B), type of radiation therapy (C) and primary histologic differentiation (D).

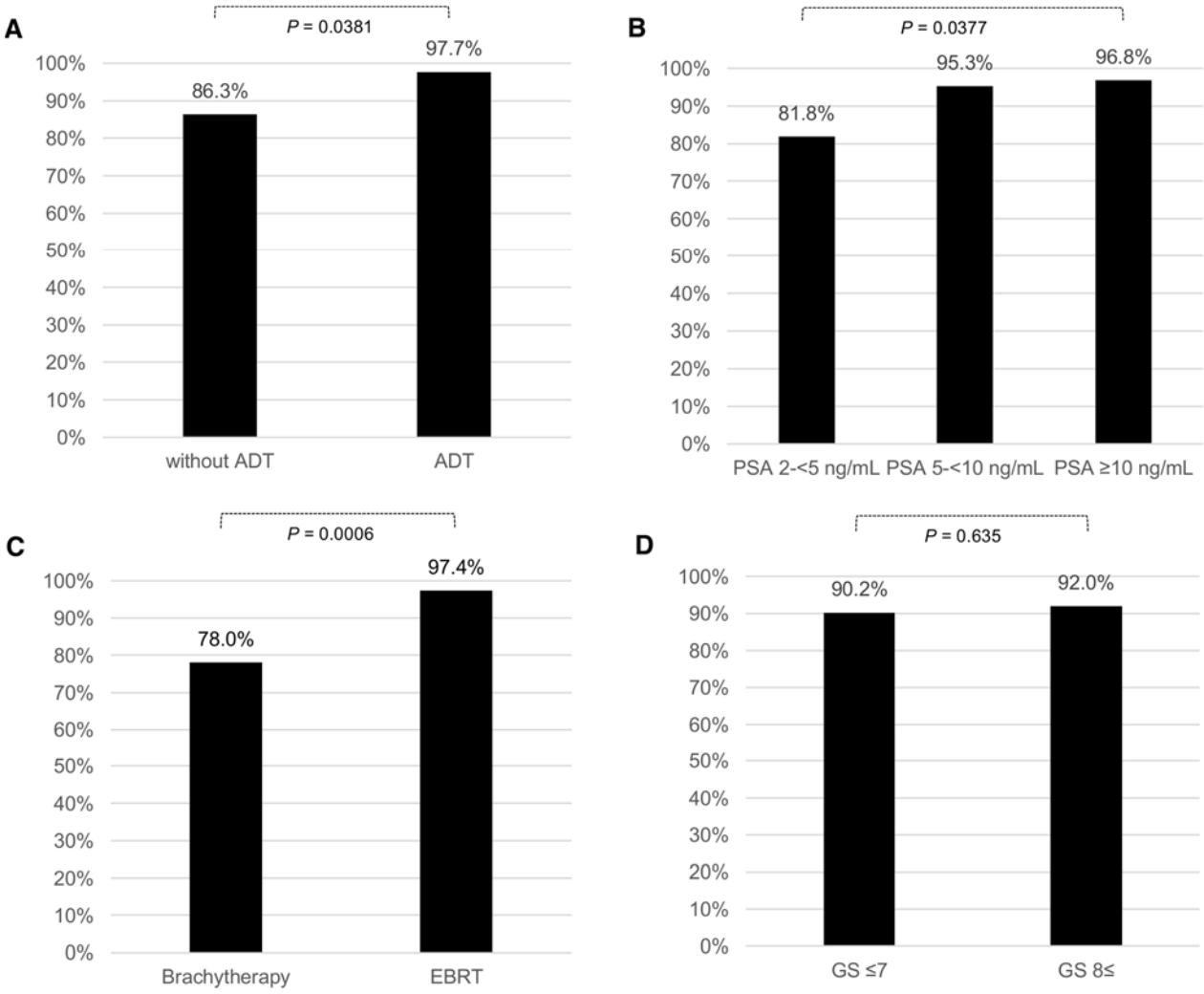


Figure 3. Distribution of ^{68}Ga-PSMA ligand PET/CT findings suspicious for recurrent prostate cancer.

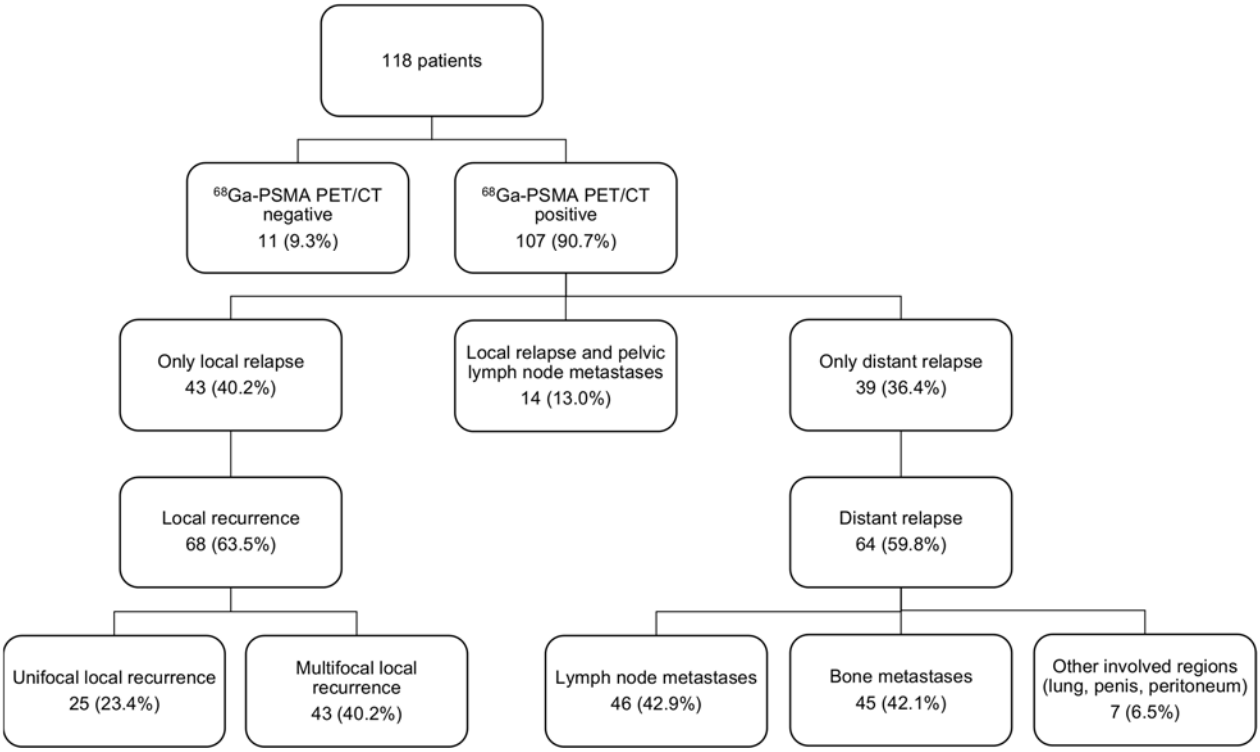


Figure 4. Multifocal local recurrence of prostate cancer in a 65-year-old patient (GS 9, PSA nadir 0.01 ng/mL after EBRT, staging PSA level 3.8 ng/mL). CT (A) was negative, whereas PET (B) and fused PET/CT images (C) revealed multiple ^{68}Ga-PSMA ligand positive lesions in the prostate gland (SUV_{max} 11.3). This finding was confirmed by transrectal ultrasonography guided sextant biopsy.

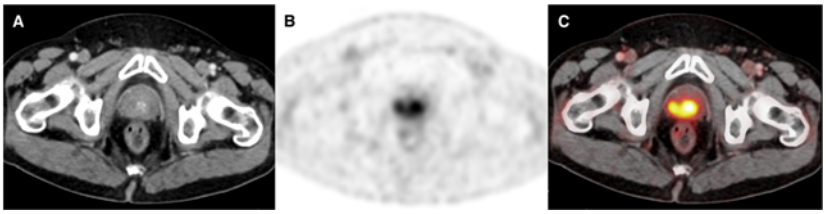


Figure 5. A 81-year-old patient with recurrent PC (GS 8, PSA nadir 0.5 ng/mL after EBRT, staging PSA level 3.34 ng/mL). CT images (A) reveal no suspicious finding in the penis. Corresponding PET (C) and fused PET/CT images (D) demonstrate high focal uptake (SUV_{max} 11.4) in the proximal part of the penis indicating soft tissue metastasis (*red arrow*). Maximum-intensity projection of the whole body (B) shows this penis metastasis and indicates in addition multifocal local recurrence (*pink arrow*), supra- and infradiaphragmatic lymph node metastases (*blue stars*) and pelvic bone metastases (*green arrows*).

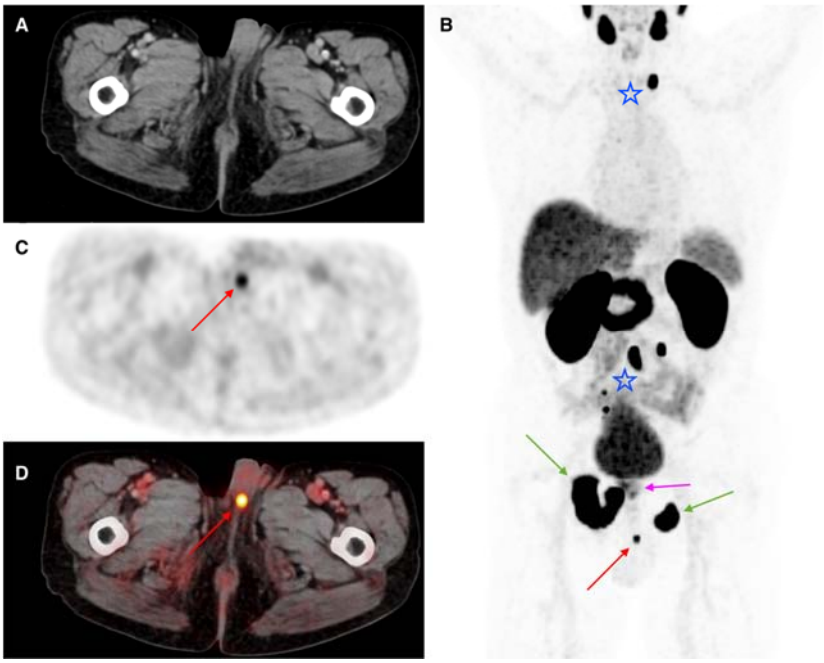


Table 1: Patient characteristics

Characteristics	n
No. of patients	118
EBRT	77
Photon therapy	63
Proton therapy	14
Brachytherapy	41
ADT during/within 6 months prior to imaging	45
Age (y)	
Median (Range, IQR)	72 (50-87, 67-76)
Primary Gleason Score*	
Median (Range, IQR)	7 (6-9, 6-8)
PSA (ng/mL)	
Median (Range, IQR)	6.4 (2.2-158.4, 4.2-10.2)
Initial PSA (ng/mL) †	
Median (Range, IQR)	10.7 (1.7-195.0, 6.9-24.7)

***† In 32/118 and 29/118 patients initial Gleason score and initial PSA remained unknown, respectively.**
IQR, interquartile range

Table 2: PSA-values in the study population considering ^{68}Ga-PSMA Ligand PET/CT results, androgen deprivation therapy, Gleason score and type of radiation therapy

Categories	PSA (ng/mL)	*P*
Positive vs. negative PET/CT findings	6.7/(2.2-158.4, 4.4-10.4) (n=107) vs. 3.8/(2.8-11.1, 3.5-5.0) (n=11)	0.0152
ADT vs. without ADT	7.7/(2.2-65.0, 4.6-15.6) (n=45) vs. 5.9/(2.2-158.4, 3.9-8.9) (n=73)	0.087
GS ≤ 7 vs. ≥ 8	7.2/(2.8-158.4, 5.1-11.0) (n=61) vs. 5.0/(2.2-25.0, 3.4-9.5) (n=25)	0.056
EBRT vs. Brachytherapy	7.0/(2.2-65.0, 4.0-11.0) (n=77) vs. 5.7/(2.6-158.4, 4.3-9.0) (n=41)	0.340

Data are median/(range, interquartile range)

Table 3: Multivariable linear regression analyses: Influence of clinical and pathological features on SUVs

IV	RC	95% CI		*P* (SUV_{max})	RC	95% CI		*P* (SUV_{mean})
		Lower	Upper			Lower	Upper	
PSA	0.011	0.002	0.020	0.018	0.011	0.001	0.020	0.025
ADT	0.486	0.162	0.810	0.004	0.465	0.133	0.797	0.007
Type of RT	-0.228	-0.589	0.133	0.212	-0.236	-0.606	0.134	0.208
Age	-0.007	-0.028	0.013	0.477	-0.007	-0.028	0.014	0.494
Injected activity	-0.006	-0.013	0.000	0.063	-0.006	-0.013	0.001	0.083
Acquisition time	0.000	-0.013	0.012	0.961	0.001	-0.012	0.014	0.886
BMI	0.009	-0.038	0.056	0.700	0.006	-0.042	0.053	0.816

IV, independent variables; RC, regression coefficient; CI, confidence interval
Incomplete data (GS and iPSA) were excluded to include more patients in this regression model (n=107)

Supplemental Text:

MATERIAL AND METHODS

Image Acquisition

PET images were obtained using ^{68}Ga-labelled HBED-CC. $^{68}Ga^{3+}$ was obtained from a ^{68}Ge/^{68}Ga radionuclide generator (iThemba Labs, Cape Town) and complexed with the HBED-CC conjugate by means of a fully automated module (Scintomics) and good manufacturing practice-grade disposable cassettes and agent kit (ABX). The final product was formulated in isotonic phosphate-buffered saline with subsequent sterile filtration. The ^{68}Ga-PSMA ligand complex was applied to patients via an intravenous bolus (mean 149±27 MBq, range 85-215 MBq, interquartile range 130-165 MBq). Variation of injected radiotracer activity was caused by the short half-life of ^{68}Ga and variable elution efficiencies obtained during the lifetime of the ^{68}Ge/^{68}Ga radionuclide generator.

PET acquisition was started at a mean time of 55.7±12.2 min after tracer injection (range: 35-81 min, ICR: 49-59 min). All patients underwent ^{68}Ga-PSMA ligand PET/CT on a Biograph mCT scanner (Siemens Medical Solutions). A diagnostic CT scan was performed first in the portal venous phase after intravenous injection of contrast agent (Imeron 300), followed by the PET scan. All PET images were acquired in three-dimensional mode with an acquisition time of 3-4 min per bed position. Emission data were corrected for randoms, dead time, scatter, and attenuation and were reconstructed iteratively by an ordered-subsets expectation maximization algorithm (4 iterations, 8 subsets) followed by a postreconstruction smoothing gaussian filter (5 mm in full width at half maximum).

Validation Criteria

Imaging findings were validated in 35.5% (38/107) of patients with PET-positive results by at least one of the following procedures: (a) transrectal ultrasound-guided biopsy in patients with suspected prostate/prostate bed relapse (n=3); (b) histological analysis after salvage radical prostatectomy and/or lymph node dissection (n=3); (c) correlative conventional imaging including contrast enhanced-CT, magnetic resonance imaging (MRI) or BS (n=2); or (d) clinical follow-up including contrast enhanced-CT, MRI, BS and repeated ^{68}Ga-PSMA ligand PET/CT or PET/MRI confirming the initial suspicious lesion(s) or showing disappearance of suspected metastatic sites after local/systemic treatment and corresponding PSA decline (n=30).

Supplemental Table 1: Univariate linear regression analyses: Influence of clinical and pathological features on SUVs

IV	RC	95% CI		*P* (SUV_{max})	RC	95% CI		*P* (SUV_{mean})
		Lower	Upper			Lower	Upper	
PSA	0.012	0.002	0.021	0.014	0.011	0.002	0.021	0.019
iPSA	0.002	-0.002	0.006	0.288	0.002	-0.002	0.006	0.228
ADT	0.538	0.224	0.852	0.001	0.520	0.199	0.840	0.002
GS	-0.461	-0.859	-0.062	0.024	-0.453	-0.860	-0.046	0.030
Type of RT	-0.280	-0.631	0.071	0.117	-0.289	-0.646	0.067	0.111
Age	0.002	-0.020	0.023	0.876	0.002	-0.020	0.023	0.884
Injected activity	-0.005	-0.011	0.001	0.133	-0.005	-0.011	0.002	0.151
Acquisition time	0.003	-0.010	0.016	0.672	0.004	-0.009	0.017	0.557
BMI	0.004	-0.039	0.047	0.855	0.002	-0.042	0.046	0.935

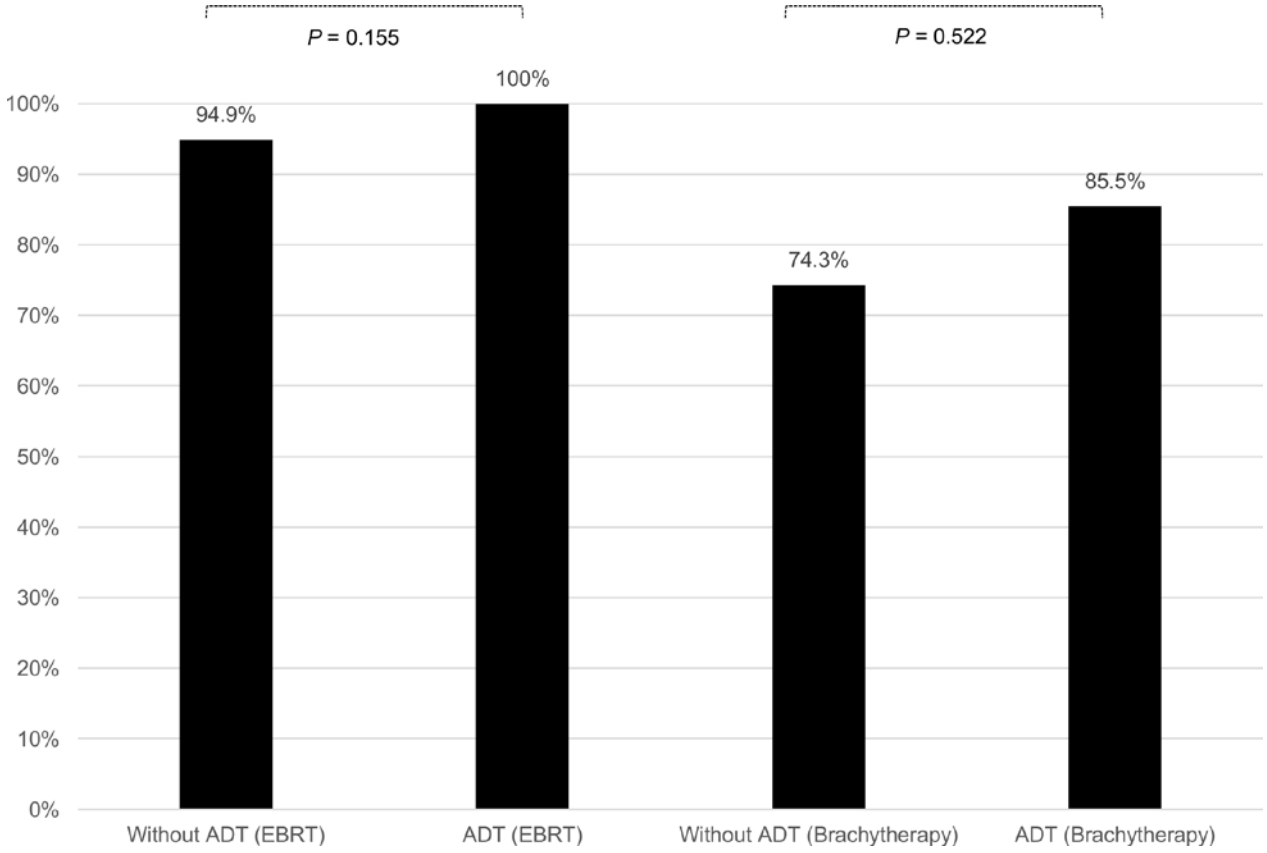
IV, independent variables; RC, regression coefficient; CI, confidence interval

Supplemental Table 2: Multivariable linear regression analyses: Influence of clinical and pathological features on SUVs

IV	RC	95% CI		*P* (SUV_{max})	RC	95% CI		*P* (SUV_{mean})
		Lower	Upper			Lower	Upper	
PSA	0.006	-0.003	0.016	0.192	0.006	-0.004	0.016	0.231
iPSA	0.001	-0.003	0.006	0.508	0.002	-0.003	0.006	0.436
ADT	0.415	-0.016	0.847	0.059	0.392	-0.049	0.834	0.081
GS	-0.121	-0.607	-0.366	0.622	-0.097	-0.595	-0.401	0.699
Type of RT	-0.387	-0.861	0.086	0.107	-0.417	-0.901	0.068	0.091
Age	0.011	-0.014	0.036	0.390	0.011	-0.014	0.037	0.379
Injected activity	-0.005	-0.013	0.003	0.219	-0.005	-0.013	0.003	0.226
Acquisition time	-0.004	-0.017	0.010	0.564	-0.003	-0.017	0.011	0.670
BMI	0.007	-0.050	0.064	0.809	0.003	-0.055	0.062	0.912

IV, independent variables; RC, regression coefficient; CI, confidence interval

Supplemental Figure 1. Detection efficacy of ^{68}Ga-PSMA ligand PET/CT in relation to androgen deprivation therapy in patients with EBRT or brachytherapy as primary radiation therapy.



Supplemental Figure 2. Set of fused PET/CT images of a 77-year-old patient with metastasized prostate cancer demonstrating ^{68}Ga-PSMA ligand positive pararectal lymph node metastasis (*white arrow; A*) and ^{68}Ga-PSMA ligand positive local recurrence (*red arrow; C*) after brachytherapy as a primary treatment (GS 7, PSA nadir below detection limit, staging PSA level 21.71 ng/mL). After subsequent chemotherapy, a follow-up ^{68}Ga-PSMA ligand PET/CT scan (B-D) did not reveal any ^{68}Ga-PSMA ligand positive suspicious finding, suggesting therapy response in accordance with a significantly decreasing PSA (0.6 ng/mL) and validating the nature of initially ^{68}Ga-PSMA ligand positive lesions as true positive.