



**Medical University
"Prof. Dr. Paraskev Stoyanov" - Varna
Faculty of Medicine
Department of Diagnostic Imaging and Radiotherapy**

Marina Dyankova, MD

**68Ga-PSMA PET/CT IMAGING IN PROSTATIC CARCINOMA.
ADVANTAGES AND POSSIBLE DIAGNOSTIC ERRORS**

Thesis Summary

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Research Supervisor: Assoc. Prof. Borislav Chaushev, MD, PhD

**Reviewers:: Prof. Anelia Klisarova, MD, DSC
Prof. Elena Piperkova, MD, DSC**

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The dissertation is presented on 197 pages, contains 3 appendices and is illustrated with 85 figures and 50 tables.

The bibliography includes 212 cited literature sources, of which 11 are in Cyrillic and 201 in Latin, the majority of them published after 2014.

The doctoral student works as an assistant professor at the Clinic of Nuclear Medicine and Metabolic Therapy at St. Marina University Hospital – Varna.

Scientific council:

Chair: Prof. Anelia Klisarova, MD, PhD, DSc.

Members:

Prof. Elena Piperkova, MD, PhD, DSc.

Prof. Irena Kostadinova, MD, PhD, DSc.

Prof. Antonia Conevska-Balcheva, MD, PhD

Prof. Dr. Marina Garcheva-Tsacheva, MD, PhD, DSc.

Prof. Anelia Klisarova, MD, PhD, DSc.

Prof. Boyan Balev, MD, PhD

Assoc. Prof. Dr. Radoslav Georgiev, Ph.D

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The materials on the defence are available in the library of MU-Varna, as well as
on the official website of the university.

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ABBREVIATIONS

ADT	-	Androgen deprivation therapy
BCR	-	Biochemical recurrence
BSc	-	Bone scintigraphy
CRPC	-	Castration-resistant prostate cancer
CT	-	Computed tomography
FDG	-	Fluorodeoxyglucose
GS	-	Gleason score
HIFU	-	High-intensity focused ultrasound
LN	-	Lymph nodes
mpMRI	-	multiparametric magnetic resonance imaging
NEDPC	-	Neuroendocrine differentiation of prostate cancer
NPV	-	Negative predictive value
PC	-	Prostate cancer
PET/CT	-	Positron emission tomography and computed tomography
PPV	-	Positive predictive value
PSA	-	Prostate-specific antigen
PSAdt	-	PSA doubling time
PSAv	-	PSA velocity
PSMA	-	Prostate-Specific Membrane Antigen
RP	-	Radical prostatectomy
RP	-	Radiopharmaceutical
RT	-	Radiation therapy
SPET/CT	-	Single-photon emission tomography/computed tomography

I. INTRODUCTION

Prostate cancer (PC) is a heterogeneous tumour with the presence of hormone-dependent and hormone-resistant populations with individual biology, prognosis and treatment. PC is the most commonly diagnosed malignancy in men worldwide. Epidemiological studies confirm the current trends of increasing morbidity and mortality of undeniable social and economic importance. Early diagnosis of PC, recurrent and metastatic lesions is crucial in determining the clinical stage, therapeutic approach, risk stratification and patient prognosis. Imaging methods are the primary means of confirming the diagnosis, referral for prostate biopsy, staging, determining the optimal treatment tactics for primary PC, as well as for re-staging in case of suspicion of recurrence after radical prostatectomy (RP) or radiation therapy (RT). Morphological imaging methods have significant limitations due to variable sensitivity and specificity. Computed tomography (CT) and magnetic resonance imaging (MRI) have low rates of detecting metastatic lymph nodes (LN), with a sensitivity ranging from 30% to 80%. Multiparametric MRI has shown promising results in diagnosing primary PC and assessing locoregional recurrence, but treatment-induced changes (anatomical distortion, fibrosis, surgical clip artefacts) may make interpretation challenging. In routine practice, bone scintigraphy (BSc) and CT are widely used to detect distant metastatic lesions, but the sensitivity of conventional methods is relatively low and has no additional diagnostic value at the low prostate-specific antigen (PSA) levels. The diagnostic value of conventional imaging methods in the early detection of PC and biochemical recurrence (BCR) often does not give satisfactory results.

The need for more sensitive and specific imaging methods for the detection of PC, early recurrence, and metastatic progression at an early stage stimulates technological advances in imaging diagnostics and particularly positron emission tomography/computed tomography (PET/CT) with various radiopharmaceuticals (RP). The hybrid PET/CT testing combines the metabolic information obtained from PET and the detailed anatomical localisation and morphological correlation provided by a CT scan, overcoming the limitations of conventional imaging methods. Recently, different RP for PET with different target structures for PC have been developed, among which ^{11}C - and ^{18}F -labeled choline have become widespread as markers of membrane metabolism and RP targeting prostate-specific membrane antigen (PSMA) – ^{68}Ga -PSMA. The high sensitivity and specificity of ^{68}Ga -PSMA PET, as well as the high resolution, allow for early detection of recurrences.

The method demonstrates a higher diagnostic value compared to the conventional imaging methods (including PET/CT-choline). PSMA-PET has a high potential for detecting early oligometastatic PC, high-frequency detection of small lesions, which allows the detection of nodal and distant metastatic lesions at an earlier stage. Hybrid imaging modality is of significant clinical importance, influencing the therapeutic approach of patients with PC.

^{68}Ga -PSMA PET/CT has been successfully used for the initial staging of high-risk primary PC, for the detection of radiologically occult metastases in LN and bone structures, although the role of the method in this context has not been well investigated. There are still discussions about the optimal imaging testing for the initial staging of the primary PC with intermediate and high risk. Published literature data from studies evaluating various potential interacting factors associated with PSMA-PET positivity in patients with BCR after radical therapy are ambiguous and, in some respects, contradictory (in terms of Gleason score). In the

studied literature, we did not find answers to recent questions concerning the indications for ^{68}Ga -PSMA PET/CT and the sensitivity (PSA-based) of the hybrid method in patients with biochemical progression after RP in the lower PSA range, including < 0.2 ng/mL. The application of the method for testing ISUP grade 5 patients is not well studied. It is necessary to conduct nuclear medical studies in Bulgaria to compare the available preliminary results and confirm the world experience in the application of the innovative for our country hybrid ^{68}Ga -PSMA PET/CT imaging method. This is necessary in order to thoroughly investigate the advantages and possible diagnostic errors of the method, optimise the criteria for selecting patients by reducing the frequency of false-negative results, as well as providing a more extensive and more reliable statistical analysis with a greater cohort of patients. The above debatable issues and problems motivated us to conduct the present study to define the role of the innovative for our country and highly promising ^{68}Ga -PSMA PET/CT method as a hybrid imaging modality in diagnosing PC, based on a comprehensive analysis of world literature and personal, professional experience.

II. AIM AND TASKS

1. AIM

To evaluate the role of ^{68}Ga -PSMA PET/CT in testing patients with PC, by investigation the advantages of the hybrid imaging method and possible diagnostic errors.

2. TASKS

1. To study the use of ^{68}Ga -PSMA PET/CT in patients with BCR of prostate cancer after radical therapy and to reveal the advantages of the method over conventional CT.
2. To determine the role of PSMA-PET in testing patients with biochemical progression after RP in a wide range of PSA (with emphasis on low tumour marker values), including PSA < 0.2 ng/mL.
3. To study the application of the method in the initial regional nodal (N) and distant metastatic (M) staging of patients with primary PC with intermediate and high risk before radical therapy; to detect possible diagnostic errors of the hybrid method, as well as to determine the advantages over CT.
4. To determine the influence of the hybrid ^{68}Ga -PSMA PET/CT method on regional nodal (N) and distant metastatic (M) staging compared to conventional imaging methods (CT, MRI and BSc) in patients with newly diagnosed histologically verified primary PC with intermediate and high risk.
5. To study the application of the method in patients with high-risk PC – International Society of Urological Pathology (ISUP) grade 5 (Gleason score 9 and 10).
6. To determine the parameters of ^{68}Ga -PSMA PET/CT: detection rate, sensitivity, specificity, accuracy, positive predictive value (PPV), negative predictive value (NPV) in different diagnostic groups of patients.

III. MATERIALS AND METHODS

For the first time in Bulgaria, the new method of nuclear medicine – ^{68}Ga -PSMA-11 PET/CT, was introduced simultaneously in the Clinic of Nuclear Medicine and Metabolic Therapy at St. Marina University Hospital – Varna, and in in Acibadem City Clinic. Our work on the application of the innovative imaging method ^{68}Ga -PSMA PET/CT in diagnosing PC includes analytical evaluation aimed at studying the specific applications of the method in different groups of patients, as well as a comparative assessment of PSMA PET/CT data compared to conventional imaging methods. The study was retrospective, with follow-up of patients, additional follow-up imaging, and histological verification of surgical interventions, performed prospectively.

1. Selection of clinical groups of patients

The patients included in our study were tested at the Clinic of Nuclear Medicine and Metabolic Therapy at St. Marina University Hospital – Varna. We selected our study group based on the inclusion criteria presented in a separate section, from a total of 837 patients with PC referred for PSMA-PET testing, and examined in the period July 2019 – Jan. 2021. We analysed retrospectively and included in the study a total of 386 patients diagnosed with PC in the period 2019 – 2021 in order to achieve adequate follow-up of patients. Information about the conducted control imaging examinations, as well as operative interventions and biopsies (histological verifications), we searched in the hospital digital storage – PACS (picture archiving and communication systems), available medical documentation of patients and hospital archives.

Criteria for inclusion and exclusion in the study and distribution of the 5 patients' groups according to tasks

2. Inclusion criteria

1. Investigating the use of ^{68}Ga -PSMA PET/CT in patients with BCR and comparing to CT, we included 133 patients (group I) with diagnosed PC and radical therapy (RP, definitive RT, as well as 3 patients with high-intensity focused ultrasound (HIFU) with suspected recurrence due to tumour marker elevation in two consecutive tests – actual PSA values ≥ 0.2 ng/mL in patients after RP, and PSA ≥ 2.0 ng/mL above nadir (lowest value after therapy) in patients after definitive RT. The following conditions were met when enrolling patients in the respective group: patients with BCR to rule out or detection of local recurrence in the prostate bed gland, regional nodal and distant metastases (nodal, bone and visceral) with PSA levels ≤ 10.0 ng/mL, as well as PSA > 10.0 ng/mL with non-categorical or negative results from conventional imaging methods (CT and/or MRI, BSc and/or SPECT/CT); patients with BCR with a short PSA doubling time (PSAdt) and an initial high Gleason score. We considered 60 days to be the optimal time range between the imaging tests (CT and PSMA-PET).

2. For assessing the role of the method in biochemical progression in patients after RP, we selected 144 patients (group II) with PC and biochemical progression after RP with persistently rising PSA values in two consecutive tests, in a wide range of tumour marker values, including low PSA levels $< 0.2 \text{ ng/mL}$ (not defined as BCR). However, this patients' cohort ($n = 70$) was identified by us as specific, and of significant clinical interest. We included also patients with persistently elevated PSA (biochemical persistence) with a persevering tendency to increase tumour marker values in two consecutive tests ($n = 10$), as well as 40 patients with BCR (with PSA levels $\geq 0.2 \text{ ng/mL}$) ($n = 64$).
3. For assessing the use of PSMA-PET in regional nodal (N) and distant metastatic (M) staging of primary PC, we included a total of 109 patients (group III) with histologically verified intermediate and high-risk primary PC (EAU risk groups for BCR of localised and locally advanced PC) before scheduling RP, RT or other radical therapy. CT testing was considered as inclusion criteria in patients with PSA levels $\geq 10.0 \text{ ng/mL}$. We considered 60 days to be the optimal time range between imaging tests.
4. To compare the diagnostic value of ^{68}Ga -PSMA PET/CT by contrast with conventional imaging methods (CT, MRI and BSc) in PC staging, we selected and included 69 patients (group IV) with histologically verified intermediate to high risk PC before scheduling any radical therapy. The patients are part of group III, referred for staging with ^{68}Ga -PSMA PET/CT. For them, conventional imaging tests were performed as well, as part of the staging protocol. In all 69 patients, ^{68}Ga -PSMA PET/CT and whole-body planar BSc were performed as inclusion criteria with a specific optimal time range of 90-days between the imaging tests.
5. To evaluate the use of PSMA-PET in patients with high-risk PC (ISUP grade 5) from patient groups I, II and III, we selected a total of 61 patients with ISUP 5 and formed group V as follows: from group I (patients with BCR) we selected 16 patients (subgroup 1); from group II (patients with biochemical progression after RP) – 22 patients (subgroup 2); from group III (patients with primary PC) – 23 patients (subgroup 3). Including criteria for the respective subgroups of patients are set out above (for group I, group II and III, respectively).
6. To determine the detection rate, sensitivity, specificity, accuracy, PPV and NPV of PSMA-PET in the different analytical patient groups, we evaluated the above parameters in I, II, III and V patients' groups.

3. Exclusion criteria for the relevant patient groups

1. Patients with biochemical progression and performed radical therapy with PSA levels $< 0.2 \text{ ng/mL}$ after RP and PSA $< 2.0 \text{ ng/mL}$ above nadir in patients after definitive RT, as well as patients with a tendency to reduced PSA values upon inclusion. Patients with missing data on the values of initial (at diagnosis) and actual PSA (up to 1 month before ^{68}Ga -PSMA PET/CT test). Patients diagnosed with synchronous/metachronous malignancy. The exclusion criterion was considered to be a CT scan performed within a time range exceeding 60 days.

2. Patients with a tendency to decreased PSA values upon inclusion. Patients with missing data on actual PSA values (up to 1 month before PSMA-PET test).
3. Patients referred for determining the site of re-biopsy with high suspicion of PC unconfirmed by the previous biopsy. Patients with missing data on the actual PSA values (up to 1 month before a staging ⁶⁸Ga-PSMA PET/CT test). Patients referred for regional nodal (N) and distant (M) staging after topical therapy.
4. As exclusion criteria were considered the lack of BSc test, conventional imaging tests (CT, MRI or BSc) conducted in another medical institution, and a time range between conventional and hybrid imaging methods exceeding 90 days.
5. Exclusion criteria for the three subgroups of patients (from group V) are described above for the respective groups: I, II and III.
6. For information regarding conducted control examinations we checked the hospital digital storage system (PACS), patients' documentation, as well as in the hospital archive. The main characteristics of the studied patients (groups V) are presented in the Results section for the respective groups of patients according to the tasks.

4. Location of the study

All nuclear medical imaging tests included in the study were conducted in the Clinic of Nuclear Medicine and Metabolic Therapy. The conventional imaging tests (CT and MRI) were conducted in the Diagnostic Imaging Clinic at St. Marina University Hospital – Varna. All clinical and laboratory tests were performed in the Clinical Laboratory at St. Marina University Hospital – Varna.

5. Research methods

5.1. Clinical methods

Medical history: For all patients with inclusive for the study indications, we compiled a detailed medical history, including data on the duration of the disease, the values of initial and actual PSA, Gleason score. In BCR cases we reviewed PSA and PSA kinetics levels; previously conducted therapy, clinical symptoms, allergies, renal failure, and other concomitant diseases. All patients were informed about the benefits and risks of the tests. After explaining the procedure, each patient was instructed in advance about the importance of not moving during the tests.

5.2. Imaging (instrumental) methods

5.2.1. PET/CT method

Devices: The research was performed on a hybrid device – Phillips Gemini TF, manufactured in 2009, combining 16-slice CT and PET in 3D mode (Fig. 1). The quality control of „Phillips Gemini TF” PET/CT was carried out according to an approved protocol for periodic control by Phillips, as per to the requirements of Ordinance № 2 of 05/02/2018 about the terms and conditions for ensuring the protection of persons in the event of medical exposure.



Figure 1. PET/CT „Phillips Gemini TF”

Radiopharmaceutical: ^{68}Ga -Glu-Urea-Lys (Ahx) -HBED-CC (PSMA-11) was synthesised in the radiochemical laboratory at the Clinic of Nuclear Medicine and Metabolic Therapy. PSMA-11 labelled with eluted ^{68}Ga is a generator product, positron emitter with a half-life of 67.63 minutes synthesised using the HBED chelator (^{68}Ga -PSMA HBED-CC or ^{68}Ga PSMA-11) according to EANM recommendations.

Generator: $^{68}\text{Ge}/^{68}\text{Ga}$ generator – iThemba LABS 50 mCi. Automated Synthesis – SCINTOMICS GRP 4V. Synthesis of ^{68}Ga -PSMA-11. Precursor: PSMA-11, manufactured by ABX GmbH Germany (ABX Advanced Biochemical Compounds (Radeberg, Germany). ^{68}Ga was transferred to a SCINTOMICS GRP 4V cassette automated synthesis module followed by acetone-free radiochemical cation exchange treatment.

^{68}Ga -PSMA-11 for i.v. administration was injected as an intravenous bolus at an activity of 1.8 – 2.2 MBq (0.049 – 0.060 mCi) per kilogram body weight (mean dose of 2 MBq (0.054 mCi) per kg body weight for whole-body scan protocol. The syringe was treated with the same saline volume (0.9% NaCl) followed by i.v. injection.

Quality control: Radiochemical yield (RCY): HPLC system with UV and gamma detector, Thin-layer chromatography (TLC). Parameters of the analytical methods – according to the national protocols and the European Pharmacopoeia. Production principles and Good Manufacturing Practice (GMP) rules and regulations were observed. The requirements for chemical, radiochemical, radionuclide purity, as well as for sterility, pyrogenicity, non-toxicity were met.

Patients' preparation: Patients had no dietary restrictions and could take the usual medications, including androgen-deprivation therapy (ADT). An intravenous route was cannulated in advance. Prior to conducting the test and during the RP accumulation, we provided good hydration to patients per os 2 hours before the scan. In order to reduce the expected high residual urinary activity in the bladder and ureters, after RP injection, the loop diuretic furosemide 20 mg i.v. was administered, except for patients with medical

contraindications to furosemide, including allergies. Patients lay in well-heated individual boxes for an average of 60 minutes (accumulation phase), after which they were asked to empty their bladders just before the scan. After positioning the device with raised hands above the head, we performed the test for about 55 – 80 minutes, recording the interval between the RP injection and the start of scanning (accumulation time). Mean accumulation time 60 ± 7 minutes (range, 55 – 80 minutes).

Test protocol

We used the Body PET/CT protocol, including native Low Dose CT 120 keV, 50 – 130 mAs, and PET in a 3D mode in the respective field with FOV-576 mm and pixel size 4 mm, 2 min for the frame from the vertex to the middle of the thighs from the bottom up. Imaging reconstruction was performed by an interactive method according to the manufacturer Body CTAC-NAC protocol: with three iterations of 36 subsets with subsequent biphasic segmentation and attenuation correction – from CT images, as well as correction of the radiation scattering and random matches.

Generating in three sets of images was presented as: uncorrected NAC, corrected PREVIEW (according to 3D-RAMLA algorithm) and corrected CTAC (BLOB-OS TF). Subsequently they were recorded in the hospital digital storage system (PACS). Daily procedures for quality control of the device were performed. The obtained images allowed visual and quantitative SUV evaluation and superimposition/fusion of CT images.

Criteria for evaluating ^{68}Ga -PSMA PET/CT in patients with PC

In all patients with performed relevant therapy, the tests were conducted at least 1 month after surgery, at least one month after chemotherapy and at least three months after RT. All patients included in the study were scanned with ^{68}Ga -PSMA PET/CT and staged according to the latest Eighth Revision of the international cancer TNM Classification. All ^{68}Ga -PSMA-11 PET/CT images were analysed and interpreted by an experienced nuclear medicine specialist with many years of expertise in hybrid PET/CT scans. The visual assessment was performed in accordance with the procedural guidelines and the latest miTNM recommendations. In the analysis of the detection rate, the suspected findings were considered positive (except for group IV, where the suspected findings were evaluated separately from the positive ones).

5.2.2. Gamma camera method

Devices: The study was performed on SPECT/CT double-headed gamma camera „MEDISO AnyScan”, manufactured in 2019 (Fig.2). The hybrid equipment has two rotating heads equipped with a low-energy general-purpose LEGP collimator. According to technical data, the spatial resolution of the system is 8.2 mm. The energy window centred on the photon energy with a peak of ^{99}mTc (140 keV) and a window width of 20%. The quality control of the „MEDISO AnyScan” SPECT/CT gamma camera was carried out according to an approved protocol for periodic control following the requirements of Ordinance № 2 of 05/02/2018 for the terms and conditions ensuring the protection of persons in the event of medical exposure.



Figure 2. Double-headed SPECT/CT gamma camera „MEDISO AnyScan”

Radiopharmaceutical: ^{99m}Tc -Methylen-diphosphonate (^{99m}Tc -MDP) for whole-body BSc was synthesised in the radiochemical laboratory at the Clinic of Nuclear Medicine and Metabolic Therapy. A radiopharmaceutical kit (MDP) was used for labelling with eluted sodium pertechnetate ($^{99m}\text{TcO}_4$) – generator product ($^{99}\text{Mo}/^{99m}\text{Tc}$ generator), gamma emitter (140.5-keV) with a half-life of 6.02 hours. ^{99m}Tc -MDP- i.v. application at 740 MBq activity (20 mCi).

Quality control of the RP: The criterion for the quality of the Russian Federation was its normal biodistribution, in compliance with all requirements: chemical, radiochemical and radionuclide purity, as well as for sterility, non-toxicity and pyrogenicity; in strict compliance with the marking procedure, the last three conditions were guaranteed by the manufacturer of the semi-finished RP.

Patients' preparation: Pre-placed venous route, good hydration per os about 1 – 1.5 litres of water after RP injection. Immediately prior to the test, the patients were instructed to empty their bladders. Scintigraphy was performed between the second and third hour of RP application (120 – 140 minutes) after positioning the patients on the scanning device.

Test protocol: Whole-body static BSc (anterior and posterior projections) was performed for all group IV patients (n = 69). For 28 patients additional targeted static and SPECT/CT scans of selected areas were performed. We conducted a whole-body planar scintigraphy according to the Whole body protocol with a 20 cm/min speed with a matrix size of 1024×256 in front and rear projections. In some patients (n = 28), we performed additional targeted static scintigraphy in front, rear and side projections with a matrix size of 128×128 or 256×256 . The conducted SPECT/CT examination was performed in a tomographic mode in a circular orbit of 360° with "Step and shoot" registration and a matrix size of 128×128 , 60 projections of 30 seconds at an interval of 3° – a total of 30 minutes with subsequent reconstruction of the images – Interactive Reconstruction.

5.2.3. Conventional imaging methods

MRI method. An MRI apparatus GE 1,5 T HDx/t Signa, 2008, product of ω Software: 15.0_M4A_0947.a Coil – 8HRBRAIN COIL was used for MRI imaging. The test was performed for 48 patients of group IV (n = 69) targeted for PC staging. Test technique: SAG T2 FS, AX T2, AX T1, COR T2, AX DWI, COR T2 FAT SAT. No contrast enhancement.

CT method. Two computed tomographs were used: Siemens Somatom Definition Dual Source – 128-slice high-resolution 4-dimensional scanner, and Siemens Spirit Somatom – spiral computed tomography. CT was performed in patients with BCR of PC (group I) and in patients

referred for staging (group III and IV), using native and/or contrast scanning. CT scans were evaluated for nodal and distant metastatic lesions, as well as for local recurrence.

Staging with conventional imaging methods in group IV patients

Prior to performing PSMA PET/CT, clinical T-stage assessment was performed with a digital rectal examination, as well as conventional imaging tests: CT, MRI and BSs. Experienced imaging specialists evaluated CT and MRI imaging results according to the current guidelines, including the size of the LN. CT images were assessed for nodal and distant metastatic lesions, and pelvic lymph node sizes > 8 mm were considered positive. Low dose CT was conducted as part of the Body PET/CT whole-body scan protocol and interpreted without overlapping of the metabolic PET imaging. Findings for regional nodal (N) and distant metastatic (M) staging by conventional imaging methods, including low-dose CT as well as PSMA PET/CT, were interpreted and categorised as: 1) negative for PC, 2) suspicious lesions that cannot be categorically defined as benign or malignant/PC, 3) positive – lesions considered as PC. Conventional imaging methods were considered complementary. Composite/aggregate data on regional nodal engagement (N status) for conventional imaging methods were obtained by grouping the CT and MRI scan results as follows: we considered a positive result in the presence of at least one positive result from conventional imaging methods, defined as dominant over negative and/or a suspicious result of the other modality; we considered a suspicious result to be dominant over the negative result of the other imaging method. Composite data for distant metastatic involvement (M staging) for conventional imaging methods were obtained by grouping the results of CT, MRI, and BSc as follows: a positive result was considered in the presence of at least one positive result from conventional imaging methods, defined as dominant compared to negative and/or suspicious result of the other modalities; we considered a suspicious result to be dominant over the negative result of the other imaging methods.

5.3. Statistical methods

Qualitative variables are represented by an absolute number and relative share in the descriptive analysis of participants' demographic and clinical characteristics. Quantitative variables were categorized by median value and standard deviation or by the median and interquartile range (IQR), depending on the type of the distribution, previously verified by the Kolmogorov–Smirnov test. The hypothesis stress test for the relationship between two qualitative variables, was evaluated by a chi-square test. The Fisher test was used to compare proportions. Spearman's rank correlation was used to evaluate the relationship between two quantities measured on an ordinal scale. Comparisons between two quantitative variables were made with Student's t-test. A comparison between more than two groups was made with one-way analysis of variance (ANOVA).

The diagnostic capabilities of PSMA-PET were determined by the proportion (detection rate) of diagnosed local recurrence and other localisations of recurrent PC, as well as for regional nodal and distant metastases during the staging of primary PC, calculated for all diagnostic groups of patients. Each indicator is presented with a 95% confidence interval. Four quadruple tables have been created to calculate: the sensitivity, specificity, the positive and negative predictive values, and the accuracy of ⁶⁸Ga-PSMA PET/CT for PC detection (separately for groups I, II, III and V). We performed a single and multiple regression analysis to establish the predictors for positive PSMA PET/CT. The multiple regression analysis models

included variables associated with a positive result supported by $p < 0.1$. Odds ratios (ORs) with 95% confidence intervals are presented.

All statistical tests are two-sided. The results are reported as statistically significant at a permissible error level $\alpha = 0.05$. The results are summarised in tables and are illustrated with appropriate graphs: box type (box top), columnar, linear, ROC curves and others. Statistical analyses were computed with the statistical package IBM SPSS ver. 21, and the graphs were built in Microsoft Excel for Windows.

IV. RESULTS AND DISCUSSION

1. ⁶⁸Ga-PSMA PET/CT in patients with BCR and comparison of the hybrid method with CT (group I)

In the researched literature, the data evaluating the various potential interacting factors associated with PSMA-PET positivity (pathological results) in scanning patients with BCR are heterogeneous and partly contradictory. Our task was to analyse the diagnostic value of PSMA PET/CT in patients with BCR after radical therapy, to determine the prognostic factors for the positivity of PSMA-PET results and the factors related to the detection rate, as well as to discover the benefits of the method compared to CT in this context.

In 79 (59.4%) patients of the group with PSA ≥ 1.0 ng/mL, CT was performed (with a time range of 60 days between scans). In 71 patients contrast-enhanced CT was conducted, in 8 patients – a CT without contrast agents due to the existing allergic reactions, in 47 patients the performed CT was of the pelvis and abdomen, in 32 – a CT scan of the pelvis, abdomen and chest was conducted. In 54 (40.6%) patients (with PSA < 1.0), the comparative assessment was performed based on Low dose CT (as part of the whole-body PET/CT protocol), given the low sensitivity of the conventional imaging method at the corresponding low PSA values.

Characteristics of patients in group I

Table 1 presents the main clinical indicators characterizing the patients in group I. Patients were distributed according to the ISUP grade as follows: 38 (28.6%) patients with ISUP 1; 34 (25.6%) – with ISUP 2; 18 (13.5%) – with ISUP 3; 27 (20.3%) – with ISUP 4; 16 (12.0%) – with ISUP 5.

Distribution of patients according to EAU risk group: 13 (9.8%) low-risk patients; 26 (19.5%) with medium risk; 94 (70.7%) at high risk for BCR (according to EAU criteria before therapy).

We divided the patients according to the initial PSA (iPSA) values as follows: 44 patients with iPSA < 10.0 ng/mL; 43 patients – (10.0- < 20.0 ng/mL); 55 – (20.0- < 50.0); 11 with iPSA ≥ 50.0 ng/mL.

Patients were also distributed according to the actual PSA (aPSA) values as follows: 20 patients with aPSA (0.2- < 0.3 ng/mL); 10 – (0.3- < 0.4); 10 – (0.4- < 0.5); 14 – (0.5- < 1.0); 17 – (1.0- < 2.0); 17 – (2.0- < 5.0); 45 – with aPSA ≥ 5.0 ng/mL.

Table 1. Characteristics of patients with BCR of PC-group I

Parameters	n (%)
Total number of patients	133
Age – yrs, median (± SD)	68.8 (8.3)
initial PSA ng/mL, median (range)	13 (3-600)
actual PSA ng/mL, median (range)	2 (0.2-638)
Gleason score, median (range)	7 (6-10)
PSAdt, median months (range)	24 (2-168)
< 6 months	28 (21.1)
6 – 12 months	15 (11.3)
> 12 months	90 (67.7)
Conducted radical therapy	
Radical prostatectomy	76 (57.1)
Definitive RT total	24 (18.1)
Brachytherapy	7 (5.3)
Intensity-modulated RT (IMRT)	17 (12.8)
Postoperative rescue RT	30 (22.6)
HIFU therapy	3 (2.2)
Clinical T stage	
T1	13 (9.8)
T2	72 (54.1)
T3	42 (31.6)
T4	6 (4.5)
Gleason score	
6	38 (28.6)
7	52 (39.1)
8	27 (20.3)
9	15 (11.3)
10	1 (0.8)
ADT	73 (54.9)

RT – radiation therapy, HIFU – high-intensity focused ultrasound, ADT – androgen-deprivation therapy, SD – standard deviation

A total of 133 patients with BCR after radical therapy were tested. RP was performed in 76 (57.1%) and rescue postoperative RT – in 30 (22.6%) patients before ⁶⁸Ga-PSMA PET/CT and CT scans. Definitive RT was performed on a total of 24 (18.1%) patients. Initial clinical T2 stage (at the time of diagnosis) was found in 72 (54.1%) patients. In 52 (39.1%) patients – Gleason score 7. In the distribution of patients according to the PSA doubling time (PSAdt), in 90 (67.7%) patients PSAdt was found to be > 12 months. In 73 (54.9%) patients a hormone therapy was performed before and/or during the PSMA-PET test (Table 1).

Detection rate and localisation of areas with pathological PSMA-activity

⁶⁸Ga-PSMA PET/CT detected recurrent PC in 90 (67.7%) patients out of 133 in whom at least one positive or suspected lesion was detected. The PSMA-PET detection rate was positively related to the level of actual PSA – with increasing the aPSA values, the detection rate of pathological PSMA-PET results increased as well (Fig. 3 and Table 2).

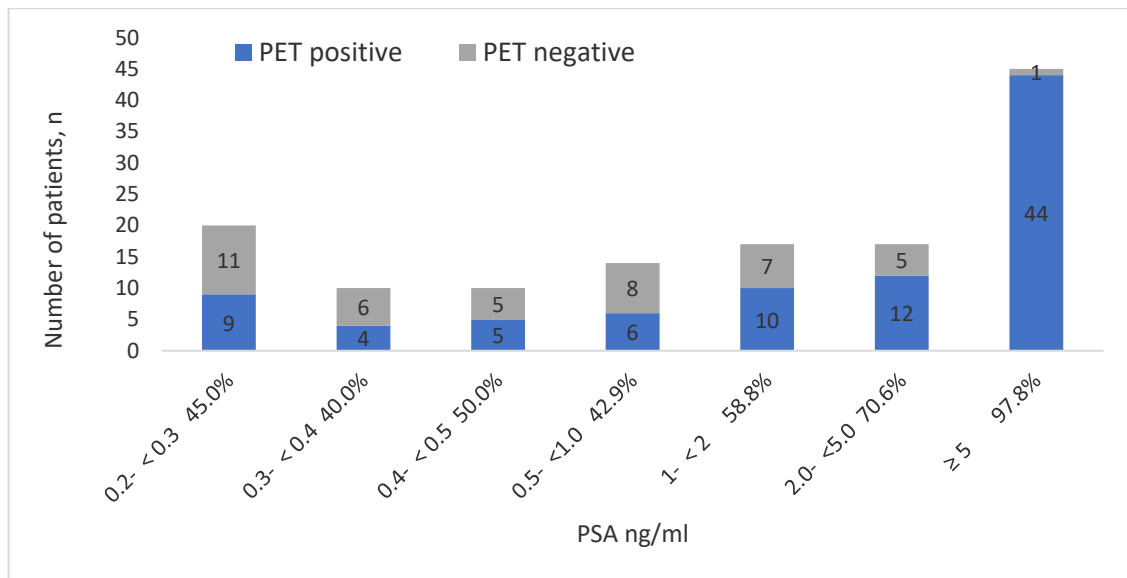


Figure 3. Distribution of PSMA-PET positive and PSMA-PET negative results for different levels of actual PSA

When determining the relationship between the aPSA level and a positive PSMA PET/CT result (results are presented in Table 2), a statistically significant relationship was found for PSA values ≥ 5.0 ng/mL ($p = 0.000$). Pathological PSMA-PET results were found in 66 (83.5%) of 79 patients with aPSA levels ≥ 1.0 .

Table 2. Correlation between actual PSA values and positive PSMA-PET score

PSA ng/ml	Позитивен ^{68}Ga -PSMA PET/CT	OR	p
<1.0	44.4% (24/54)	1.0	
1.0 <5.0	64.7 % (22/34)	2.29	0.060
≥ 5.0	97.8% (44/45)	55.0	0.000

The detection rate was not related to the PSAdt ($p > 0.05$) values: a slightly higher detection rate was found in patients with PSAdt < 6 months compared to PSAdt 6 – 12 months and PSAdt > 12 months (Table 3).

Table 3. Distribution of PSMA-PET positive results relative to PSAdt

PSAdt, month	Patients, n (%)	Positive PET/CT, n (%/%*)
< 6	28 (21.1)	22 (16.5/ 78.6)
6-12	15 (11.3)	11 (8.3/ 73.3)
> 12	90 (67.7)	57 (42.9/ 63.3)

PSAdt- PSA doubling time, %* - percentage of all patients in the group ($n = 133$) / percentage of patients in the respective subgroup

We found a statistically significant positive correlation between the detection rate and the levels of initial PSA (Table 4). As iPSA values increased, the detection rate also increased, with the highest values (90.9%) at nPSA ≥ 50.0 ng/mL.

Table 4. Correlation between initial PSA values and positive PSMA-PET score

Initial PSA ng/mL	Positive ⁶⁸ Ga-PSMA PET/CT	OR	p
< 10.0	50.0% (22/44)	1.0	
10.0 < 20.0	65.1 % (28/43)	1.9	0.156
20.0 < 50.0	54.5% (30/55)	6.0	0.002
≥ 50.0	90.9% (10/11)	10.0	0.035

In the distribution of positive PSMA PET/CT results compared to ISUP grade, there was a tendency to increase the detection rate related to an increasing ISUP grade: 55.2% positive PSMA-PET at ISUP grade 1, 64.7% – at ISUP grade 2, 72.2% – at ISUP grade 3, 74.0% for ISUP grade 4, 87.5% for ISUP grade 5, but without a statistically significant relationship.

In the distribution of positive PSMA PET/CT results compared to the initial clinical T stage, we also reported a tendency of increased detection rate with increasing the T stage: 64.2% – at T1, 55.6% – at T2, 85.4% – at T3 + T4.

We reported a tendency of increased detection rate when increasing the risk group for BCR, but without a statistically significant relationship (Table 5).

Table 5. Correlation between EAU risk group and positive PSMA–PET results

EAU Risk	Positive ⁶⁸ Ga-PSMA PET/CT	OR	p
Low risk	46.2 % (6/13)	1	
Medium risk	57.7 % (15/26)	1.59	0.497
High risk	73.4 % (69/94)	3.22	0.053

EAU – European Association of Urology

Areas with pathologically active ⁶⁸Ga-PSMA findings were analysed for conducted radical therapy and location of the recurrence (Table 6).

Table 6. Localisation of recurrent PC (patient-based) in BCR by ⁶⁸Ga-PSMA PET/CT scan

Conducted radical therapy	Patients, n	Local recurrence, n (%)	LN, n (%)	Bone, n (%)	LN and bones, n (%)	Visceral, n (%)	PSMA positive, n (%)
Radical prostatectomy	76	26 (34.2)	19 (25.0)	13 (17.1)	3 (3.9)	3 (3.9)	44 (57.9)
Definitive RT	24	13 (54.2)	9 (37.5)	13 (54.2)	5 (20.8)	2 (8.3)	21 (87.5)
Postoperative rescue RT	30	2 (6.7)	9 (30.0)	16 (53.3)	3 (10.0)	3 (10.0)	22 (73.3)
HIFU therapy	3	2 (66.7)	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)	3 (100.0)
Total n (%)	133	43 (32.3)	37 (27.8)	43 (32.3)	11 (8.2)	8(6.0)	90 (67.7)

RT – radiation therapy, HIFU – high-intensity focused ultrasound, LN – lymph nodes

The most common localisations were found for: local recurrence – 43 (32.3%) and bone metastases 43 (32.3%). Visceral metastases were found in 8 (6.0%) patients, of which 7 patients had lung metastases, and one patient had liver metastases. When evaluating the detection rate of recurrent PC compared to different subgroups of patients with relevant radical therapy, no statistically significant association was found ($p > 0.05$). The highest indicators were reported in patients after HIFU-3 (100.0%), followed by those with definitive RT – 21 (87.5%). Local recurrence was most common in patients after HIFU-2 (66.7%) and definitive RT – 13 (54.2%); malignant involvement of the LN in patients with definitive RT – 9 (37.5%), followed by those

with rescue RT – 9 (30%); bone metastases were found most often in patients after definitive RT – 13 (54.1%), as well as after rescue postoperative RT– 16 (53.3%), in whom bone involvement was most common, while in only 2 (6.7%) patients we found a local recurrence.

Parameters related to pathological PSMA PET/CT results

When evaluating the parameters related to pathological PSMA PET/CT results (Table 7 A, B), we analysed the potential influence of several factors: age, ISUP grade, initial clinical T stage, actual PSA, ADT, as well as Gleason score (separately calculated), using single-variant and multiple regression analysis. From the performed single-variant analysis we found the following predictors for PSMA-PET positivity: ISUP grade ($p = 0.024$), aPSA ($p = 0.02$), T stage ($p = 0.016$), ADT ($p = 0.05$), and Gleason score ($p = 0.024$). After multivariate regression analysis, only aPSA values were derived as a significant predictive factor for PSMA positivity ($p = 0.002$).

Table 7. Results from the single-variant and multivariate analysis of the parameters associated with a positive ^{68}Ga -PSMA PET/CT result

Characteristics	Positive PSMA-PET, % (n)	Negative PSMA-PET, % (n)	single-variant analysis			multiple analysis		
			OR	95% CI	p	OR	95% CI	p
Age	69.03 ±8.53	68.3 ±7.87	1.010	0.98-1.06	0.655			
ISUP group								
1	55.3 (21)	44.7 (17)	1.650	1.07-2.55	0.024	1.376	0.805-2.253	0.243
2	64.7 (22)	35.3 (12)						
3-5	77 (47)	23.0 (14)						
PSA (ng/ml)	25.8 (77.9)	1.12 (1.69)	1.440	1.15-1.81	0.002	1.430	1.14-1.79	0.002
Clinical T stage								
T1	69.2 (9)	30.8 (4)	2.120	1.15-3.89	0.016	2.061	0.922-4.608	0.078
T2	55.6 (40)	44.4 (32)						
T3 и T4	85.4 (41)	14.6 (7)						
ADT								
No	55.0 (33)	45.0 (27)	0.340	0.16-73	0.005	0.788	0.318-1.948	0.605
Yes	78.1 (57)	21.9 (16)						
Gleason score								
6	55.3 (21)	44.7 (17)	1.74	1.07-2.828	0.024	1.303	0.715-2.377	0.243
7	67.3 (35)	32.7 (12)						
8-10	79.1 (34)	20.9 (14)						

Comparison of actual PSA values and PSAdt in patients with a positive versus negative PSMA-PET results

When comparing the actual PSA values for patients with a positive versus negative PSMA-PET result, we found a significant difference ($p = 0.039$) (Table 8). However, the data for PSAdt presented slightly faster kinetics in positive versus negative PSMA-PET scans ($p = 0.839$).

Table 8. Values of actual PSA and PSA kinetics (PSAdt) in ^{68}Ga -PSMA PET/CT positive and negative patients

	^{68}Ga -PSMA PET/CT	Patients, n	Median	SD	Median	Range	p
PSA (ng/ml)	Positive	90	25.89	77.92	4.155	0.2-638	0.039
	Negative	43	1.12	1.69	0.472	0.2-9.83	
PSAdt (months)	Positive	90	39.69	39.02	24	2-144	0.839
	Negative	43	41.19	41.23	26	3-168	

PSA – prostate-specific antigen, PSAdt – PSA doubling time

Detection rate in localisation of PC recurrence versus the limit values of aPSA < 0.5 ng/mL and PSAdt – 6 months

When estimating the detection rate at different localizations of recurrence compared to the limit values for PSAdt – 6 months, for aPSA < 0.5 ng/mL we found slightly higher values of 78.6% for PSAdt < 6 months compared to 68.4% for PSAdt \geq 6 months (Table 9). We reported a detection rate of 45.0% for PSA < 0.5 ng/mL versus 77.4% for PSA \geq 0.5 ng/mL. The highest detection rate – 85.7%, was found in PSA > 0.5 and PSAdt < 6 months. Pathological results were found in 4 (57.1%) of 7 patients with PSA < 0.5 ng/mL and PSAdt < 6 months, in all of whom local recurrence was found, as well as a higher frequency of bone metastases – 57.1%. Detection rate was higher at 85.7% (18/21) in patients with PSA \geq 0.5 ng/mL and PSAdt < 6 months, local recurrence was found in 7 (33.3%) patients with metastatic LN, bone metastases – in 7 (33.3%), metastatic LN and bone metastases – 1 (4.8%), visceral metastases – 1 (4.8%). PSMA-PET was positive in 14 of 33 patients (42.4%) with PSA < 0.5 ng/mL and PSAdt \geq 6 months.

Table 9. PSMA-PET detection rate of malignant lesions using PSAdt limit values of 6 months and actual PSA of <0.5 ng/mL

PSA, ng/mL PSAdt, month.	Patients, n	detection rate, %			Bone	LN and bones	Visceral
		Total	Local recurrence	LN			
PSAdt < 6	28	78.6	25	32.1	39.3	8	4
PSAdt \geq 6	105	68.4	34.3	26.7	30.5	8.5	4
PSA < 0.5	40	45	17.5	25	25	7.5	0
PSA \geq 0.5	93	77.4	38.7	29	36.6	8.6	5.3
PSA < 0.5 PSAdt < 6	7	57.1	100	28.6	57.1	14.3	0
PSA < 0.5 PSAdt \geq 6	33	42.4	21.2	24.2	18.2	6	0
PSA \geq 0.5 PSAdt < 6	21	85.7	33.3	33.3	33.3	4.8	4.8
PSA \geq 0.5 PSAdt \geq 6	72	75	40.3	27.8	36.1	9.7	5.5

PSAdt – PSA doubling time, PSA – prostate-specific antigen

Comparative analysis of the ⁶⁸Ga-PSMA PET/CT and CT results

The frequency of CT detection for local recurrence was reported at 6 (4.5%), for metastatic LN (regional and distant) – 17 (12.8%), for bone metastases – 23 (17.3%), in 8 (6.0%) patients were found visceral metastases, of which 5 with unconvincing suspected data. The results of the comparative analysis of ⁶⁸Ga-PSMA PET/CT and CT in assessing the different areas of PC involvement, as well as the values of aPSA (median, range) in patients with established recurrence only with PSMA-PET/CT, are presented in Table 10. In 37 (86.0% of patients out of 43 with the respective localisation), local recurrence was detected only with PSMA-PET, without suspected CT data. Patients with metastatic LN detected with PSMA-PET only, without any CT suspicions (n 20 /54.0% of 37 patients with the respective localisation) were due to the CT limitations in assessing structural changes (LN size); mean value, mm ± SD/median (range). When measuring the short diameter of the LN, we calculated 5.97 ± 1.66/6 (3.0–8.0) mm, while the corresponding values in the measurement of the involved LNs detected by both methods were 16.41 ± 4.52 / 17 (10.0 – 27.0) mm. Bone metastases with PSMA-PET alone were found in 20 (46.5% of patients out of 43 with the corresponding localisation), and in all of them, the secondary lesions were due to bone marrow involvement (without morphological pathologies). In the assessment of ⁶⁸Ga-PSMA PET/CT > CT for the respective localisation of the malignant involvement, cases with additional lesions detected by PSMA-PET were not included, i.e. patients with at least one suspected CT finding.

Table 10. ⁶⁸Ga-PSMA PET/CT > CT of different locations of recurrent PC detected only with PSMA-PET (patient-based assessment) at corresponding values of actual PSA (median, range)

⁶⁸ Ga-PSMA PET/CT > CT	Patients, n	%/%*	PSA, median (ng/mL)	PSA, range (ng/mL)
Local recurrence	37	86.0/ 27.8	5.15	0.21 – 212.2
Metastatic LN	20	54.0/ 15.0	5.85	0.22 – 638.1
Bone metastases	20	46.5/ 15.0	5.14	0.2 – 84.4

PSA-prostate-specific antigen, *%/%/Relative share of positive patients with respective localisation/Relative share of all patients in group I

⁶⁸Ga-PSMA PET/CT is significantly superior to CT in detecting recurrent lesions, especially for the assessment of local recurrence. Additional PSMA-PET positive findings were found in 36 (40%) of 90 positive patients when evaluated for additional metastatic lesions.

In conclusion, ⁶⁸Ga-PSMA PET/CT has a high detection rate of recurrent PC in patients with BCR after radical therapy, even at low actual PSA values, with an overall detection rate of 67.7% and 45.0% in PSA < 0.5 ng/mL, similar to the results of the retrospective study by Afshar-Oromieh et al. The frequency of PSMA-PET detection in the present study was positively related to aPSA levels, similar to the results of other published studies. The most common localisations were local recurrence and bone metastases (32.3%). Local recurrence is most common in patients after HIFU – 66.7% and definitive RT – 54.2%; malignant involvement of the LN in patients with definitive RT – 37.5%; metastatic bone lesions in patients after definitive RT – 54.1%, and rescue postoperative RT – 53.3% (no statistically significant correlation), which is in line with the data from a recent prospective study. Significant differences were found in aPSA values with positive versus negative PSMA-PET score (p = 0.039), similar to those published in other studies. Detection rate of recurrent lesions is positively related to the level of aPSA and iPSA. The ISUP grade, initial clinical T stage and

EAU risk groups and ADT tend to have a similar relationship. There is a significant increase in the frequency of PSMA-PET detection with increased iPSA values: 54.5% at 20.0- < 50.0 ng/mL, and 90.9% at iPSA \geq 50.0 ng/mL, and for actual PSA levels \geq 5.0 ng/mL. The detection rate of local recurrence was higher (100.0%), as well as for bone metastases (57.1%) in PSA < 0.5 ng/mL, PSAdt < 6 months. However, the detection rate did not show a significant correlation with PSAdt values, similarly to a recently published study. In the analysis of the studied parameters (age, ISUP grade, Gleason score, aPSA, T stage and ADT) and their correlation to a pathological PSMA PET/CT score, only aPSA values were derived as a significant predictive factor for PSMA-positivity (multiple regression analysis), which is in line with the results of recently published studies. PSMA PET/CT significantly exceeds conventional CT in detecting recurrent lesions, especially for the assessment of local recurrence – 86.0%, for metastatic LN – 54.0%, for bone metastases – 46.5% (detection with PSMA-PET only). Additional PSMA-active lesions were found in 40% of the positive patients. Our results are consistent with the data by Eiber et al.

2. ^{68}Ga -PSMA PET/CT in patients with biochemical progression after RP focused on low PSA levels, including < 0.2 ng/mL (group II)

In the literature research, we did not find answers to current questions about the indications and sensitivity (based on PSA) of the hybrid PSMA-PET method in patients with rising PSA levels after RP (biochemical progression) in the lower range of PSA values, including < 0.2 ng/mL. Whether an increase in PSA is indicative of local recurrent PC or metastatic disease, as well as a correlation to the Gleason score, remains important. Our task was to analyse the influence of PSA values in the biochemical progression of sensitivity and the detection rate of PSMA PET/CT, as well as to determine the relationship of detection rate of regional or metastatic PC involvement in patients after RP and the impact of several parameters (Gleason score, PSA, ADT) on the probability of detecting a pathological ^{68}Ga -PSMA PET/CT result.

Characteristics of the patients in group II

The main clinical indicators characterising group II patients are presented in Table 11. Total number of patients (n = 144). The mean age of patients (\pm SD) was reported at 67.3 (\pm 8.3) years.

Table 11. Characteristics of patients with post-RP biochemical progression over a wide range of PSA values, including < 0.2 ng/mL (group II)

Parameters	n (%)
Total number of patients	144
Age, mean age (\pm SD)	67.3 (8.3)
PSA, median (range)	0.233 (0.02-497)
PSA, median (\pm SD)	11.03 (52.28)
Gleason score, median (range)	7 (6-10)
Gleason score	
6	40 (27.8)
7	67 (46.5)
8	14 (9.7)
9	19 (13.2)
10	4 (2.8)
ADT	52 (36.1)

ADT – Androgen deprivation therapy, SD – standard deviation

Calculated mean PSA (\pm SD) – 11.03 (\pm 52.28) ng/mL, Gleason score, median (range) – 7 (6 – 10). Gleason score 7 was found in 67 (46.5%). ADT/hormone therapy before and/or during the PSMA PET/CT study was conducted in 52 (36.1%) patients.

The distribution of patients in six subgroups relative to the PSA values is shown on Figure 4. More than 1/2 of the patients (group II) are presented with PSA levels \leq 0.5 ng/mL. We included patients with post-RP biochemical progression with PSA < 0.2 ng/mL (a specific, clinically significant cohort with low PSA values below the BCR-defining levels) and patients with BCR, as described above.

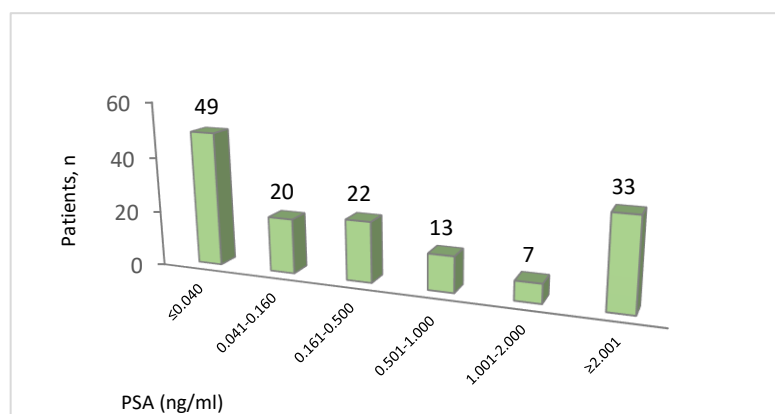


Figure 4. Distribution of patients by PSA value in the 6 PSA-subgroups

We assessed the correlation between the PSA levels in the six subgroups of patients and the detection rate, the sensitivity of ^{68}Ga -PSMA PET/CT (based on PSA), and the detection rate of regional or metastatic PC recurrence. We determined the mean (\pm SD) of PSA at which local, regional, and systemic PC recurrence was detected, the influence of several parameters (Gleason score, PSA, conducted ADT) on the probability of detecting pathological PSMA-PET,

and Gleason score correlation with the detection rate for different recurrence PC localisations, ADT and PSA.

We found a pathological ^{68}Ga -PSMA-11 PET/CT result in 62 (43.1%) of a total of 144 patients presenting at least one positive lesion or suspected PC recurrence. The detection rate results in relation to the localisation of recurrent PC lesions are presented in Table 12. The highest detection rate was reported for distant metastases in total (75.8%) – in 47 of 62 patients with pathological results.

Table 12. Detection rate relative to recurrent PC localisation in ^{68}Ga -PSMA PET/CT scans of patients with biochemical progression after RP

	n (% /%)*
Total number of patients	144
PSMA-PET positive patients Total	62 (43.1)
Local recurrence	19 (13.2/30.6)
Regional metastatic LN	15 (10.4/24.2)
Distant metastatic LN	18 (12.5/29.0)
Bone metastases	39 (27.1/ 62.9)
Distant metastases Total	47 (32.6/ 75.8)
Regional metastatic LN and distant metastases	12 (8.3/ 19.4)

% /% * – percentage of positives for the respective localization of recurrence in relation to the total number of patients (n = 144) / Percentage of positives for the respective localization in relation to positive PSMA-PET patients (n = 62)

The hybrid method detected recurrent PC in 10 patients with a PSA level below 0.2 ng/mL (16.1% of 62 patients with pathological outcomes). The results for the localisation of recurrent PC versus PSA values in the 6 subgroups of patients are presented in Table 13 A – the relative share of positives for the respective localisation is presented in relation to the total number of patients in the respective PSA subgroup. We found a statistically significant correlation between the PSA levels and the frequency of PSMA-PET detection (p < 0.001). The detection rate was reported to be the highest for the 6th subgroup (94.0%).

Table 13. A. Localisation of recurrent PC versus PSA values in the 6 subgroups of patients in ^{68}Ga -PSMA PET/CT scans

PSA subgroups of patients PSA (ng/mL)	Patients, n (%)	Local recurrence, n (%)	Regional LN, n (%)	Distant LN, n (%)	Bone n (%)	Distant metastases Total, n (%)	Local recurrence and regional	PSMA positive, n (%)
1) ≤ 0.04	49 (34)	0 (0.0)	1 (2.0)	2 (4.1)	5 (10.0)	6 (12.0)	0 (0.0)	6 (12.0)
2) $> 0.04- 0.16$	20 (13.9)	0 (0.0)	0 (0.0)	1 (5.6)	3 (15.8)	3 (15.8)	0 (0.0)	3 (15.8)
3) $> 0.16- 0.50$	22 (15.3)	3 (13.6)	3 (13.6)	0 (0.0)	8 (36.4)	8 (36.4)	1 (4.5)	11 (50.0)
4) $>0.50 - 1.00$	13 (9.0)	3 (23.1)	1 (7.7)	3 (23.1)	2 (15.4)	4 (30.8)	0 (0.0)	7 (53.8)
5) $>1.00 - 2.00$	7 (4.9)	1 (14.3)	2 (28.6)	3 (42.9)	1 (14.3)	3 (42.9)	1 (14.3)	4 (57.1)
6) > 2.00	33 (22.9)	12 (36.4)	8 (24.2)	9 (27.3)	20 (60.6)	23 (69.7)	3 (9.1)	31 (94.0)
p value		p < 0.001	P = 0.012	P = 0.001	p < 0.001	p < 0.001	p < 0.001	p < 0.001

Table 13 B presents results for the localisation of recurrent PC compared to the PSA values in the 6 subgroups of patients – the relative share of the positives for each localisation was calculated relative to the total number of positive patients for the respective PSA subgroup.

Table 13. B. Localisation of recurrent PC by ⁶⁸Ga-PSMA PET/CT scan versus PSA values in the 6 subgroups of patients

PSA subgroups of patients PSA (ng/mL)	Local recurrence, n (%)*	Regional metastatic LN, n (%)	Distant metastatic LN, n (%)	Bone metastases, n (%)	Distant metastases Total, n (%)
1) ≤ 0.04	0 (0.0)	1 (17.0)	2 (33.3)	5 (83.3)	6 (100.0)
2) > 0.04- 0.16	0 (0.0)	0 (0.0)	1 (33.3)	3 (100.0)	3 (100.0)
3) > 0.16- 0.50	3 (27.3)	3 (27.3)	0 (0.0)	8 (72.7)	8 (72.7)
4) > 0.50 - 1.00	3 (42.9)	1 (14.3)	3 (42.9)	2 (28.6)	4 (57.1)
5) > 1.00 - 2.00	1 (25.0)	2 (50.0)	3 (75.0)	1 (25.0)	3 (75.0)
6) > 2.00	12 (38.7)	8 (25.8)	9 (29.03)	20 (64.5)	23 (74.2)
p value	p < 0.001	p = 0.012	p = 0.001	p < 0.001	p < 0.001

LN – lymph nodes, % * – relative share of positive patients in the respective PSA subgroup

Local recurrence was found in patients with higher PSA values (> 0.16 ng/mL) with the highest frequency for subgroup 4 (42.9%) (Table 13 B). We found the highest detection rate for regional metastatic LN (50.0%) and distant metastatic LN (75.0%) in the 5th subgroup of patients. A higher detection rate for bone metastases was reported in patients with lower PSA values, in the first 3 subgroups, in all positive patients from subgroup 2, and in 5 (83.3%) of a total of 6 positive patients from PSA subgroup 1 (Figures 5 a, b). Distant metastatic lesions, distant metastatic LN, bone and visceral metastases in general, were most common in patients with lower PSA levels (including all positive patients from the first two subgroups) (Table 13 B).

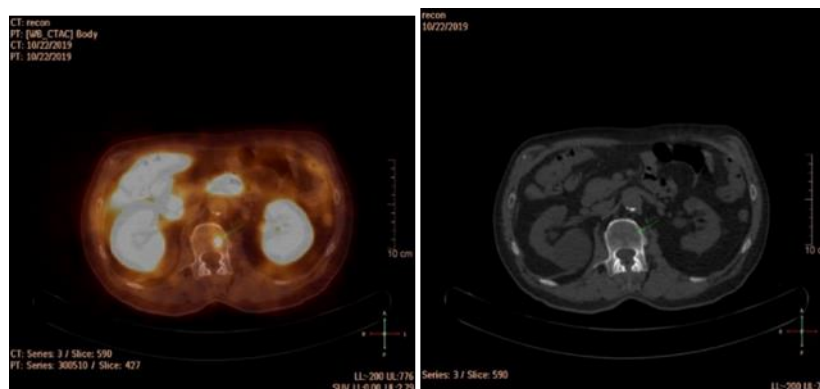


Figure 5. (a) fusion PET/CT Figure 5 (b) low dose CT axial

Figure 5 shows a 74-year-old patient with oligometastatic PC detected after radical prostatectomy with actual PSA values of 0.035 ng/mL. Gleason score 7 (4 + 3).

Figure 5 (a) ⁶⁸Ga-PSMA PET/CT fusion image representing focally intensively increased radiopharmaceutical activity in the lumbar vertebral body L1, without corresponding (correlated) low-dose CT lesions. The imaging was interpreted as a metastatic involvement at the bone marrow level. (b) Low dose CT image without established correlated lesions. The results for the detection rate with PSMA-PET at different localisations of recurrent PC are presented on Figure 6. We found a statistically significant correlation between the PSA level and the detection rate of recurrent PC in all studied localisations, p < 0.05 (Table 13, Fig. 6).

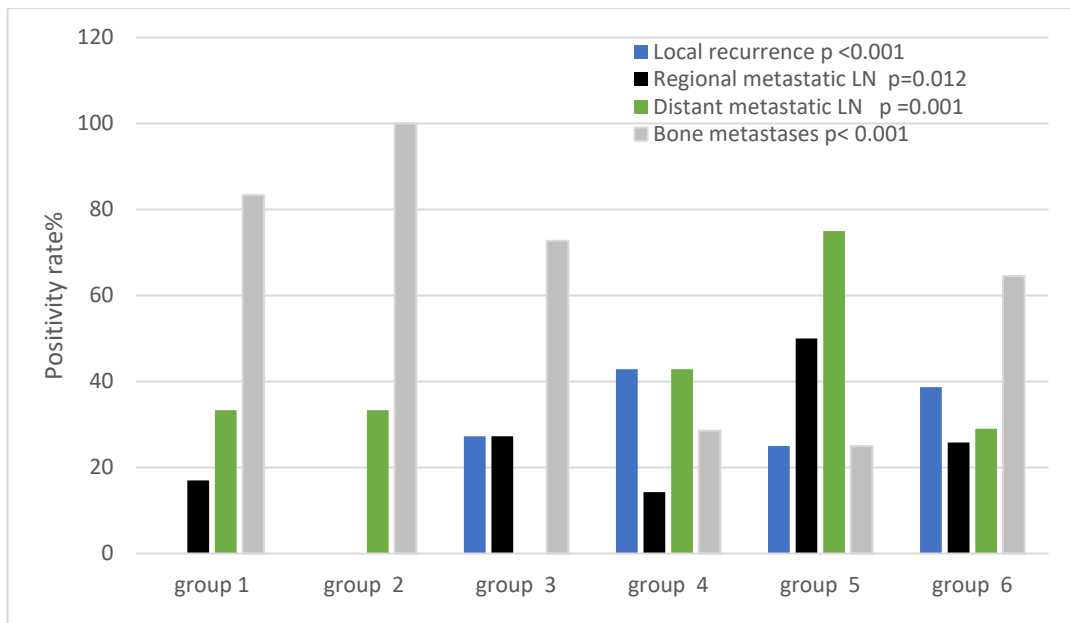


Figure 6. The relationship between PSA levels in the six PSA-subgroups and location of PC recurrence

Our calculations for the general sensitivity and specificity of the method (based on PSA levels), showed values of 58.0% and 87.0%, respectively. A sensitivity of 15.0% was calculated at the lower PSA values (0.041 - 0.160 ng/mL) for subgroup 2. In the other subgroups with higher PSA levels the sensitivity indicators were higher: 3) 50.0%; 4) 53.0%; 5) 57.0%; 6) 93.0%, respectively. Given the study's design, it was not possible to derive sensitivity data for the first subgroup of patients with PSA \leq 0.040 ng/mL.

The correlation between the presented characteristics and a positive ^{68}Ga -PSMA PET/CT result is presented in Table 14. The single-variant regression analysis found a statistically significant correlation between the Gleason score and a positive PSMA-PET result ($p = 0.001$). We reported an increase in the detection rate with an increasing Gleason score. A significant difference was found when comparing the PSA values in patients with a positive versus a negative PSMA-PET result ($p = 0.004$). We did not find a significant correlation for ADT/hormone therapy ($p = 0.83$).

Table 14. Correlation of different parameters with a positive PSMA-PET result

Characteristics	Positive PSMA-PET, n (%)	Negative PSMA-PET, n (%)	Single-variant analysis		
			OR	95% CI	P
Gleason score					
6	11 (27.5)	29 (72.5)	6.198	2.303-16.682	0.001
7	26 (38.8)	41 (61.2)	3.584	1.512-8.493	
8-10	25 (69.4)	11 (30.6)	0.44		
PSA (ng/mL) Median value (SD)	25.2 (77.77)	0.28 (0.68)			0.004
ADT					
No	39 (42.4)	53 (57.6)			0.83
Yes	23 (44.2)	29 (55.8)			

ADT – androgen deprivation therapy, SD – standard deviation

Data on PSA, ng/mL (median, SD) for the respective localisation of recurrent PC, as well as data on conducted ADT, are presented in Table 15. Statistically significant difference was found for regional metastatic LN ($p = 0.008$), bone metastases ($p = 0.0001$), as well as distant metastases in total ($p = 0.001$). In contrast, the difference was not significant for local recurrence, distant metastatic LN, as well as in the assessment of ADT ($p > 0.05$).

Table 15. Correlation between the localisation of recurrent PC and conducted ADT with PSA levels

Characteristics	PSA (ng/mL) median value (\pm SD)	p value
ADT	9.9 (34.5)	
Without ADT	11.7 (60.2)	0.846
Local recurrence	30.9 (113.16)	
No local recurrence	8.0 (34.9)	0.075
Regional metastatic LN	44.8 (126.4)	
No regional metastatic LN	7.1 (34.0)	0.008
Distant metastatic LN	16.6 (34.7)	
No distant metastatic LN	10.4 (54.8)	0.643
Bone metastases	37.6 (96.2)	
No bone metastases	1.2 (3.6)	0.0001
Distant metastases in total	75.37 (85.4)	
No distant metastases	0.7 (1.7)	0.001

ADT – androgen deprivation therapy, SD – standard deviation

In 52 (36.1%) of all patients in group II, ADT was performed before the PET/CT scan. In 23 (44.2%) patients, we found a pathological PSMA-PET result. Our results showed no significant correlation between a conducted ADT and a positive result. There was a slight difference in PSA in ADT patients compared to patients without hormone therapy. The correlation between the Gleason score and the localisation of recurrent PC, as well as a conducted ADT, and PSA values, is presented in Table 16.

Table 16. Correlation between the Gleason score and detection rate, corresponding recurrent PC localisation, conducted ADT and PSA values (in the 6 subgroups of patients)

	GS 6 n (%)	GS 7, n (%)	GS 8, n (%)	GS 9- 10, n (%)	p value
Patients, n (%)	41 (28.4)	67 (46.5)	14 (9.7)	22 (15.3)	
Positive PSMA-PET, n (%).	11 (17.7)	26 (41.9)	8 (12.9)	17 (27.5)	0.002
ADT	4 (17.4)	8 (34.8)	4 (17.4)	7 (30.4)	0.001
Local recurrence	7 (36.8)	6 (31.6)	4 (21.1)	2 (10.5)	0.272
Regional metastatic LN	1 (6.7)	7 (46.7)	1 (6.7)	6 (40.0)	0.046
Distant metastatic LN	1 (5.5)	9 (50.0)	2 (11.2)	6 (33.3)	0.0001
Bone metastases	5 (12.8)	15 (38.5)	5 (12.8)	14 (35.9)	0.0001
Distant metastases in total	5 (10.6)	20 (42.6)	5 (10.6)	17 (36.2)	0.0001
PSA (ng/mL)					0.05
1) ≤ 0.040 ng/mL	1 (9.1)	4 (15.4)	0 (0)	1 (5.9)	
2) 0.041 - 0.160 ng/mL	1 (9.1)	2 (7.7)	0 (0)	0 (0)	
3) 0.161 - 0.500 ng/mL	2 (18.2)	3 (11.5)	2 (25.0)	4 (23.5)	
4) 0.501 - 1.0 ng/mL	1 (9.1)	5 (19.2)	0 (0)	1 (5.9)	
5) 1.001 - 2.00 ng/mL	1 (9.1)	2 (7.7)	0 (0)	1 (5.9)	
6) > 2.0 ng/mL	5 (45.5)	10 (38.5)	6 (75.0)	10 (58.8)	

GS – Gleason score, ADT – androgen deprivation therapy, LN – lymph nodes

Patients with a positive PSMA-PET score – 62 (43.1%), were distributed in subgroups according to the Gleason score (GS) and the parameters with a calculated relative share of the positive scores for the respective subgroups. Pathological PSMA-PET results in GS-6 were found in 11 (26.8%) of 41 patients with the highest frequency detection of local recurrence – in 7 (63.6%) of 11 patients, with a positive result, as well as higher PSA values (subgroup 6th). Pathological results were found in 4 (36.4%) of 11 patients with ADT. Pathological PSMA-PET results in GS-7 were found in 26 (38.8%) of 67 patients with the highest detection rate for distant metastases in total – 20 (76.9%) of 26. The same refers and for the 6th PSA subgroup. Pathological results were found in 8 (33.3%) of 24 patients with ADT. PSA levels > 2.0 ng/mL were reported in 16 (48.5%) patients with high GS-8-10. In 8 (57.1%) of 14 patients with GS-8, we found a pathological PSMA-PET result with the highest detection rate for bone metastases and distant metastases in total – in 5 (62.5%) of 8, as well as for the 6th PSA subgroup. Pathological PET/CT results were found in 4 (57.1%) of 7 patients with conducted ADT (higher incidence than GS-6 and GS-7).

Pathological PSMA-PET results were found in 17 (77.3%) of 22 patients with GS 9-10. Distant metastases were reported in all 17 patients with a positive PET/CT result, in 14 of whom bone metastases were detected. Higher PSA values > 2.0 ng/mL (subgroup 6th) were found in 10 (58.8%) of 17 patients with positive results. In 7 (70.0%) of 10 patients with conducted ADT we found pathological results, and the detection rate was significantly higher than in GS-6, GS-7 and GS-8. Our results showed a significantly higher detection rate of distant metastatic lesions in total in patients with GS (8-10) – 22/36 (61.1%) compared to patients with GS (6-7) – 25/108 (23.1%), $p = 0.0001$. Slightly higher detection rate for local recurrence was found in patients with GS (8-10) – 6/36 (16.7%) compared to GS (6-7) – 13/108 (12.0%), $p = 0.272$. Patients with GS-7 also showed a higher detection rate – 26/67 (38.8%) compared to patients with GS-6 – 11/41 (26.8%), $p = 0.002$. For distant metastases in total: in GS-7 – 20/26 (76.9%) compared to GS-6 – 5/11 (45.5%), $p = 0.0001$. The association between the GS and local recurrence detection was statistically insignificant ($p = 0.272$), with a higher detection rate in patients with GS-6 – 7/11 (63.6%) compared to GS-7 – 6/26 (23.1%).

When evaluating the association of the GS (in the respective subgroups) with the various parameters, a statistically significant correlation was found with the detection rate of positive PSMA-PET results ($p = 0.002$), regional metastatic LN ($p = 0.046$), distant metastatic LN, bone and distant metastases in total ($p = 0.0001$), conducted ADT ($p = 0.001$) and PSA levels in PSMA-PET positive patients ($p = 0.05$), without significant correlation with local recurrence (Table 16).

In conclusion, distant metastatic lesions are the most commonly diagnosed site of recurrent PC in patients with biochemical progression after RP (75% of positive patients). As the tumour marker levels increase, the detection rate (positive correlation) increases, reaching 94.0% for PSA > 2.0 ng/mL, similar to data from a recent study. PSA values in patients with positive PSMA-PET results are significantly higher than in negative PSMA-PET results.

A statistically significant difference in PSA values was found in the assessment of patients with regional metastatic LN ($p = 0.008$), bone metastases ($p = 0.0001$), and distant metastases in total ($p = 0.001$). Local recurrence was found in patients with higher PSA values > 0.16 ng/mL. The detection rate is higher for regional and distant metastatic LN at PSA > 1.0 – 2.0 ng/mL. Biochemical progression after RP is more commonly associated with bone metastases in patients with lower PSA values ≤ 0.5 ng/mL. The detection rate for distant metastatic lesions

(in general) is higher at $PSA \leq 0.16$ ng/mL. PSMA-PET detected recurrent PC in 16.1% of positive patients with PSA below 0.2 ng/mL. However, the PSA levels did not reach the definition of BCR, which correlates with data from other studies. Sensitivity values are higher at higher PSA levels (93.0% for $PSA > 2.0$ ng/mL), similar to recent studies. Although the PSA levels ($> 0.04 - 0.16$ ng/mL) are below the BCR-defining values, the sensitivity of the method reaches 15.0%. The obtained results suggest a future change in the BCR definition to lower PSA values. Our results showed a significant correlation between the Gleason score and the pathological PSMA-PET result.

The detection rate of distant metastatic lesions is related to higher GS: 61.1% in GS (8-10) compared to 23.1% in GS (6-7), $p = 0.0001$, which is consistent with the results reported in the study of Hofman et al., 2019. We found a statistically significant correlation between GS and the detection of regional metastatic LN ($p = 0.046$); distant metastatic LN, bone metastases and distant metastases in total – ($p = 0.0001$), as well as detection of pathological results in patients with conducted ADT ($p = 0.001$). The PSA values in the studied subgroups/positive PSMA-PET results ($p = 0.05$) did not detect a significant correlation to local recurrence, similar to data from a recent study.

3. Application of ^{68}Ga -PSMA PET/CT in initial regional (N) and distant (M) staging of primary PC before radical therapy (group III)

There are still discussions about the indications for conducting a hybrid PSMA test in intermediate-risk patients, as well as about the factors associated with nodal and distant metastasis in primary PC. With the wider use of PSMA-PET in PC assessment, the number of published studies reporting various benign tumours and malignant neoplasms (other than PC) with increased PSMA expression has also increased. Our study aimed to investigate the application of PSMA PET/CT for initial regional nodal (N) and distant metastatic (M) staging of primary PC with intermediate and high risk before scheduling radical therapy. We aimed to identify possible diagnostic errors of the hybrid imaging method, as well as to determine the advantages of PSMA-PET over conventional CT. In the present study, we also analyse the correlation between PSA levels, clinical T stage, EAU (European Association of Urology) risk groups, and the ISUP (International Society of Urological Pathology) grade, and the detection rate of metastatic lesions at different sites. In 60 (55.0%) patients, we performed contrast-enhanced CT: in 34 (31.2%) – CT scans of the pelvis and abdomen, in 26 (23.9%) – CT of the pelvis, abdomen and chest. In 49 (45%) patients, the comparative assessment was performed based on low-dose CT as part of the whole-body scan protocol for Body PET/CT, given the lower PSA values (below 10.0 ng/mL) in these patients.

Characteristics of the studied patients in group III

Table 17 presents the main clinical indicators characterising the patients in group III, referred for staging with ^{68}Ga -PSMA PET/CT.

Table 17. Characteristics of patients targeted for staging with ^{68}Ga -PSMA PET/CT – group III

Parameters	n (%)
Total number of patients	109
Age, median yrs. (\pm SD)	68.3 (7.43)
PSA ng/mL, median (range)	12.5 (1-29)
Gleason score, median (range)	7 (6-10)
Gleason score	
6	20 (18.3)
7	41 (37.6)
8	25 (22.9)
9	15 (13.8)
10	8 (7.4)
EAU risk group	
Intermediate risk	15 (13.8)
high risk	94 (86.2)
ADT/ hormone therapy	8 (7.3)

EAU – European Association of Urology, ADT – androgen deprivation therapy, SD – standard deviation

The mean age (\pm SD) of patients in group III was 68.3 (\pm 7.43) years. Calculated median (range) of PSA ng/mL 12.5 (1 – 29) Gleason score, median (range) – 7 (6 – 10). During the PSMA-PET scan ADT was performed in only 8 (7.3%) of 109 patients referred for staging. Only 15 (13.8%) patients were with intermediate risk (EAU risk group), while 94 (86.2%) were at high risk. (Table 17).

Distribution of patients according to the ISUP (International Society of Urological Pathology) grade: ISUP 1 – 20 (18.3%), ISUP 2 – 23 (21.1%), ISUP 3 – 18 (16.5%), ISUP 4 – 25 (22.9%), ISUP 5 – 23 (21.1%). In 48 (44.0%) of 109 patients ISUP grade 4–5 was established, while in ISUP grade 1 – in 20 (18.3%) patients. Clinical T stage data were based on MRI results in 54 (49.5%) of 109 patients studied, as well as PSMA PET/CT in the remaining 55 (50.4%) patients. We distributed the patients according to the clinical T stage as follows: cT1 – 11 (10.1%), cT2a – 11 (10.1%), cT2b – 11 (10.1%), cT2c – 48 (44.0%), cT3a – 8 (7.3%), cT3b – 15 (13.8%), cT4 – 5 (4.6%), with the highest relative proportion for cT2c clinical stage.

We distributed the patients according to the PSA values as follows: in 49 (44.9%) patients the PSA levels were reported < 10.0 ng/mL, in 20 (18.3%) patients the established PSA levels were between 10.0 and 20.0 ng/mL, and in 40 (36.7%) of patients – PSA levels > 20.0 ng/mL.

Primary PC was detected with increased PSMA activity in 108 (99.1%) of 109 patients. When evaluating the primary tumour, only one patient (1 (0.9%) with histologically verified primary tumour (PC) did not show increased PSMA expression during initial staging with PSMA PET/CT. In patients at intermediate risk (n = 15), only 1 (6.6%) patient had metastatic lesions (localised in the regional LN), compared with 40 (42.6%) patients with high-risk PC (n = 94). Oligometastatic disease (\leq 5 metastatic lesions) was found in 7 (6.4%) of all patients in the group (n = 109). Regional metastatic LN were found in 29 (26.6%) patients. Distant metastatic LNs were detected in 14 (12.8%) patients. Bone metastatic lesions were found in 23 (21.1%) patients, of which 8 (7.3%) had disseminated bone metastases. Visceral metastatic

lesions were found in 4 (3.7%) patients, and distant metastases (overall) – in 31 (28.4%) patients.

The distribution of the positive ⁶⁸Ga-PSMA PET/CT results at the different localisations of the metastatic lesions of the primary PC according to the EAU risk group are presented in Table. 18. Metastatic lesions localised to regional LNs were found in only one intermediate-risk patient. As the risk group increased, the detection rate of metastatic lesions also increased. We found a statistically significant correlation between the detection rate of PC metastases and the EAU risk group for regional LN (p = 0.047) and distant metastases in total (p = 0.017).

Table 18. Distribution of positive ⁶⁸Ga-PSMA PET/CT results at different localisations of metastatic lesions in primary PC according to the EAU risk group

EAU risk group	Number of patients, n	Regional LN, n (%)	Distant LN, n (%)	Bone, n (%)	Visceral, n (%)	Distant total, n (%)
Intermediate risk	15	1 (6.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
High risk	94	28 (29.8)	14 (14.9)	23 (24.5)	4 (4.3%)	31 (32.9)
p value		0.047	0.278	0.098	0.763	0.017

EAU- European Association of Urology, LN – lymph nodes

The distribution of the positive ⁶⁸Ga-PSMA PET/CT results at different localisations of the metastatic lesions in primary PC according to the PSA values is presented in Table. 19. The detection rate of metastatic lesions (risk of metastasis) increased with increasing PSA values for regional LN (from 10.4% for PSA < 10.0 ng/mL to 50.0% for PSA > 20.0 ng/mL) and distant LN from 2.0% to 32.5% for the respective PSA values), bone metastases (from 12.2% to 37.5%), as well as for disseminated bone, visceral and distant metastases in total. This is in contrast to the oligometastatic disease detected mainly at lower PSA values.

We found a statistically significant correlation between the detection rate of PC metastases and PSA values for the various localisations specified in the table, except for oligometastatic (p = 0.799) and visceral metastasis (p = 0.055). The oligometastatic disease was detected mainly in patients with lower PSA values: in 4 of 7 patients with oligometastatic lesions, PSA values < 10.0 ng/mL were detected. In all 4 patients with detected visceral metastatic lesions, PSA levels were > 20.0 ng/mL.

Table 19. Distribution of positive ⁶⁸Ga-PSMA PET/CT results at different localisations of metastatic lesions in primary PC according to PSA values

PSA, ng/mL	Patients, n	Oligo metastases, n (%)	Regional LN, n (%)	Distant LN, n (%)	Bone, n (%)	Disseminated bone, n (%)	Visceral, n (%)	Distant total, n (%)
<10.0	49	4 (8.2)	5 (10.4)	1 (2.0)	6 (12.2)	0 (0.0)	0 (0.0)	7 (14.3)
10.0-20.0	20	1 (5.0)	4 (20.0)	0 (0.0)	2 (10.0)	1 (5.0)	0 (0.0)	2 (10.0)
>20.0	40	2 (5.0)	20 (50.0)	13 (32.5)	15 (37.5)	7 (17.5)	4 (10.0)	22 (55.0)
p value		0.799	<0.001	<0.001	0.006	0.006	0.055	<0.001

LN – lymph nodes

The distribution of the positive PSMA-PET results at the different localisations of the metastatic lesions from the primary PC according to the clinical T stage is presented in Table.

20. We did not find a statistically significant correlation between the detection rate of PC metastases and clinical T stage. We noted a tendency for a similar relationship for distant metastatic LN ($p = 0.083$). With increasing the T stage, the detection rate of positive results increased from 9.1% for cT1 to 25.0% for cT3-4. For regional LN the detection rate increased from 9.1% for cT1 to 35.7% for cT3-4. For distant metastases in total the detection rate increased from 27.3% to 42.9%, respectively.

Table 20. Distribution of positive ^{68}Ga -PSMA PET/CT results at different localisations of metastatic lesions from primary PC according to the clinical T stage

Clinical T stage	Patients, n	Oligo metastases, n (%)	Regional LN, n (%)	Distant LN, n (%)	Bone, n (%)	Disseminated bone, n (%)	Visceral, n (%)	Distant total, n (%)
cT1	11	1 (9.1)	1 (9.1)	1 (9.1)	3 (27.3)	1 (9.1)	1 (9.1)	3 (27.3)
cT2	70	4 (5.7)	18 (25.7)	6 (8.6)	11 (15.7)	6 (8.6)	2 (2.9)	16 (22.9)
cT3 -4	28	2 (7.1)	10 (35.7)	7 (25.0)	9 (32.1)	1 (3.6)	1 (3.6)	12 (42.9)
p value		0.899	0.302	0.083	0.172	0.674	0.623	0.352

LN – lymph nodes

The distribution of the positive ^{68}Ga -PSMA PET/CT results at the different localisations of the metastatic lesions of the primary PC according to the ISUP grade is presented in Table 21. With the increase of the ISUP grade, the detection rate of metastatic lesions increased, especially for regional LN (from 5.0% for ISUP 1 to 48.9% for ISUP 4-5), as well as for distant metastases in total (from 5.0% to 52.1%, respectively). We found a statistically significant correlation between the detection rate of PC metastases and the ISUP grade for regional and distant metastatic LN ($p < 0.001$), bone metastases ($p = 0.004$) and distant metastases in total ($p < 0.001$).

Table 21. Distribution of positive ^{68}Ga -PSMA PET/CT results at different localisations of metastatic PC lesions according to the ISUP grade

ISUP grade	Patients, n	Oligo metastases, n (%)	Regional LN, n (%)	Distant LN, n (%)	Bone, n (%)	Disseminated bone, n (%)	Visceral, n (%)	Distant total, n (%)
ISUP 1	20	0 (0.0)	1 (5.0)	1 (5.0)	1 (5.0)	1 (5.0)	0 (0.0)	1 (5.0)
ISUP 2-3	41	3 (7.3)	5 (12.2)	0 (0.0)	5 (12.2)	1 (2.4)	0 (0.0)	5 (12.2)
ISUP 4-5	48	4 (8.3)	23 (48.9)	13 (27.1)	17 (35.4)	6 (12.5)	4 (8.3)	25 (52.1)
p value		0.423	<0.001	<0.001	0.004	0.175	0.337	<0.001

ISUP- International Society of Urological Pathology, LN – lymph nodes

Oligometastatic disease (≤ 5 metastatic lesions) was found in 7 (6.4%) of 109 patients in the group, of which 4 (3.7%) patients with PSA level < 10.0 ng/mL and 4 (3.7%) with ISUP grade 4-5 (Table 19 and Table 21). Regional metastatic LN were found in 29 (26.6%) patients, of which 20 (18.3%) with PSA level > 20.0 ng/mL and 23 (21.1%) with ISUP grade 4-5. Distant metastatic LNs were detected in 14 (12.8%) patients, of whom 13 (11.9%) with PSA levels > 20.0 ng/mL, 8 (7.3%) with clinical stage T 3-4 and 13 (11.9%) with ISUP 4-5. Bone metastases were detected in 23 (21.1%) patients, of whom 15 (13.8%) with PSA levels > 20.0 ng/mL, and 17 (15.6%) with ISUP grade 4-5. Disseminated bone metastases were detected in 8 (7.3%), of

which 8 (7.3%) patients with PSA levels > 20.0 ng/mL, 6 (5.5%) with ISUP 4-5. Visceral metastatic lesions were found in 4 (3.7%) patients with established PSA values > 20.0 ng/mL, as well as ISUP grade 4-5. Distant metastases (in total) were found in 31 (28.4%) patients (most often in patients with higher PSA values, as well as with ISUP 4-5), of which 22 (20.2%) with PSA levels > 20.0 ng/mL and 25 (22.9%) with ISUP grade 4-5.

The detection of metastases from the primary PC was positively related to the EAU risk group, PSA and ISUP grade values. Our results showed a significantly higher detection rate of metastatic lesions in the high-risk patients (42.6%) than in the intermediate-risk patients (6.6%). With increasing PSA values and ISUP grade, the detection rate of metastatic lesions for regional and distant LN, bone, visceral and distant metastases in general increased.

Possible diagnostic errors in the interpretation of ⁶⁸Ga-PSMA PET/CT results

All cases with false-positive findings expressing PSMA as well as false-negative findings with increased PSMA activity (and verified PC) were evaluated for possible diagnostic errors (at lesion level) in ⁶⁸Ga-PSMA PET/CT staging in patients with histologically confirmed primary PC. In a total of 23 (21.1%) of 109 patients, false-positive and/or false-negative findings were found in the regional (N) and (M) staging with hybrid imaging method. False-positive findings were found in 15 (13.8%) of all patients in group III (n = 109). The distribution of false-positive findings with increased PSMA expression in the PSMA-PET staging scan is presented in Table. 22.

Table 22. Distribution of false-positive findings with increased PSMA expression in ⁶⁸Ga-PSMA PET/CT staging scans of primary PC

False-positive findings with increased PSMA expression	Cases, n (%)
Bone fractures	1 (0.9%)
Paget's disease	1 (0.9%)
Vertebral hemangioma	1 (0.9%)
Visceral hemangiomas (hepatic and lienal)	2 (1.8%)
Celiac ganglion	3 (2.8%)
Inflammatory activity in paraaortic LN	1 (0.9%)
Benign thyroid nodules (follicular adenoma)	2 (1.8%)
Synchronous primary lung cancer	3 (2.8%)
Synchronous primary colorectal adenocarcinoma of the sigmoid colon	1 (0.9%)

Synchronous primary lung cancer was found in 3 patients and synchronous primary colorectal adenocarcinoma of the sigmoid colon – in one patient.

Figures 7 – 9 illustrate some of the false-positive findings detected with ⁶⁸Ga-PSMA PET/CT during the staging of patients with primary PC. Figure 7 shows a 60-year-old patient diagnosed with primary acinar PC involving seminal vesicle/cT3b, Gleason score 8, ISUP grade 4, PSA values – 183.0 ng/mL. From the performed PSMA PET/CT staging, in addition to the PC-related lesions, we also found an accidental PSMA-active finding in the right lobe of the thyroid gland. The axial ⁶⁸Ga-PSMA PET/CT fusion image (a), as well as the Low dose CT image (b), illustrate a thyroid nodule with a 36/26 mm heterodense structures (green arrow) with increased RP activity of the fusion PET/CT image with SUVmax 5.9. Following fine-

needle aspiration biopsy (FNAB), the described finding was histologically confirmed as follicular adenoma of the thyroid gland.

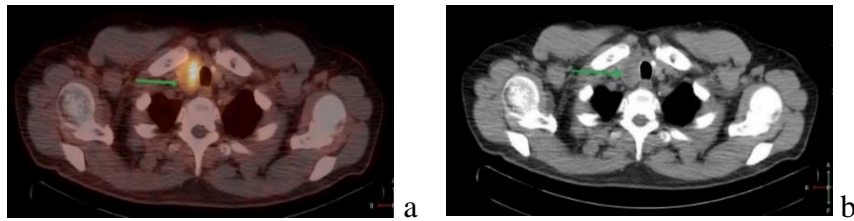


Figure 7. (a) fusion, (b) low dose CT axial

Figure 8 presents a 73-year-old patient with histologically verified primary PC – cT2c, Gleason score 7, ISUP grade 3, PSA values – 34.13 ng/mL. From the performed PSMA-PET, in addition to the detected increased PSMA expression in the primary tumour, we found a tumour formation in the upper lobe of the left lung measuring 34/30 mm with increased PSMA activity.

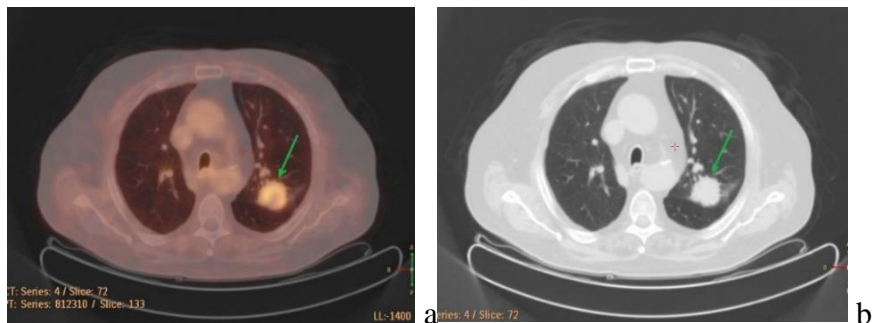


Figure 8. (a) fusion, (b) low dose CT axial

The axial ^{68}Ga -PSMA PET/CT fusion image (a) as well as the Low dose CT image (b) visualised the lung finding (green arrow) with increased fusion image activity. During follow-up it was histologically verified as synchronous primary squamous cell lung cancer. Figure 9 shows a patient with a detected PC, Gleason score 6, PSA – 75.0 ng/mL, referred for staging with PSMA-PET imaging.

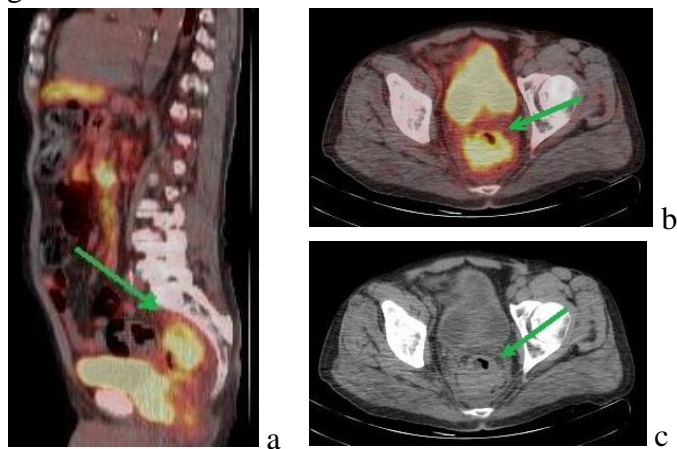


Figure 9. (a) fusion sagittal, (b, c) fusion axial, low dose CT

From the hybrid PET/CT staging test, in addition to the primary prostate tumour, we found PSMA-expressing tumour formation involving the rectum and propagating presacral, as

well as ascites fluid in the pelvis (Figure 9. a – c). Fibroendoscopy, biopsy and histology verified moderately differentiated adenocarcinoma of the rectum.

False-negative findings were found in 8 (7.3%) of all patients in group III (n = 109). The distribution of false-negative findings not expressing PSMA antigen is presented in Table 23.

Table 23. Distribution of false-negative findings not expressing PSMA antigen in ⁶⁸Ga-PSMA PET/CT staging scans for primary PC

False-negative findings not expressing PSMA antigen	Cases, n (%)
Primary PC with increased activity	1 (0.9%)
Metastatic LN without increased activity	1 (0.9%)
Pulmonary metastases	1 (0.9%)
Liver and peritoneal metastatic lesions	1 (0.9%)
Bone sclerotic metastatic lesions	2 (1.8%)
Bone osteolytic metastases	2 (1.8%)

Figure 10 presents a 62-year-old patient referred for ⁶⁸Ga-PSMA PET/CT staging due to histologically verified primary PC-cT4 (involving the bladder and rectum), Gleason score 8, ISUP grade 4, with actual PSA – 193.0 ng/mL. Increased PSMA expression in the primary tumour, pelvic and abdominal lymphadenopathy was detected from PSMA-PET scan. In addition, PSMA PET/CT detected many omental, peritoneal metastatic lesions, but without increased radiopharmaceutical activity, in places difficult to be distinguished from the high physiological intestinal activity – presented on the axial ⁶⁸Ga-PSMA PET/CT fusion images (a, b).

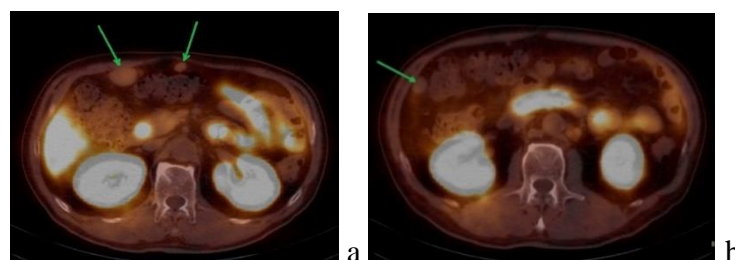


Figure 10. (a,b) fusion axial

Numerous small hypodense lesions in both liver lobes, difficult to be distinguished on the Low dose CT image (c), as well as a larger one with a size of 17 mm – did not show significantly increased PSMA expression against the background of physiologically increased activity in the liver parenchyma (d). However, they were suspected, tracked, and confirmed as metastatic PC lesions.

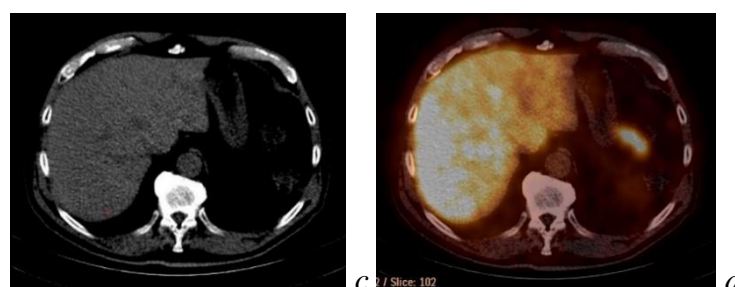


Figure 10. (c) Low dose CT, (d) Fusion axial

Comparative analysis evaluating the hybrid ⁶⁸Ga-PSMA PET/CT method versus CT for detecting metastatic lesions of primary PC in the initial staging of intermediate and high risk patients before scheduling a radical therapy

To determine the advantages of PSMA-PET over conventional CT, the cases of detection of additional metastatic LN, bone and visceral metastases (patient-based) were analysed. The comparative analysis results of the PSA values are presented in Table 24. PSMA PET/CT detected additional metastatic LN in 26.6% of the studied patients, as well as bone metastases in 15.6% of the patients. With increasing PSA values, the cases of detection of additional metastatic lesions also increased: for metastatic LN the detection rate of additional LN increased from 10.2% for PSA values < 10.0 ng/mL to 52.5% for PSA > 20.0 ng/mL; for bone metastases, the detection rate for additional lesions increased from 6.1% for PSA values < 10.0 ng/mL to 30.0% for PSA > 20.0 ng/mL.

Table 24. *⁶⁸Ga-PSMA PET/CT > CT for detection of additional metastatic LN and bone metastases corresponding to the PSA values in primary PC staging*

PSA, ng/mL	Patients, n (%)	Metastatic LN	Bone metastases
< 10.0 ng/mL	49	5 (10.2)	3 (6.1)
10.0 – 20.0 ng/mL	20	3 (15.0)	2 (10.0)
> 20.0 ng/mL	40	21 (52.5)	12 (30.0)
P value		< 0.001	0.006

LV – lymph nodes

We found a statistically significant correlation between the detection rate of additional metastatic lesions and the PSA values for LN ($p < 0.001$) and bone metastases ($p = 0.006$). With increasing the PSA levels, the detection rate of additional metastatic lesions also increased (LN and bone metastases): for LN from 10.2% for PSA <10.0 ng/mL to 52.5% for PSA > 20.0 ng/mL; for bone metastases from 6.1% to 30.0% for the corresponding PSA values. The mean value, mm \pm SD, when measuring the short diameter of the additional LNs detected only with PSMA PET/CT, we calculated 6.14 ± 1.38 , median (range) 6.0 (4 – 9) mm. In 2 of 4 patients with detected visceral metastases, contrast-enhanced CT results were positive. Additional visceral lesions were detected with PSMA PET/CT in 3 patients. PSMA-PET is significantly superior to CT in detecting metastatic LN and bone metastases in the initial staging of primary PC in intermediate and high risk patients.

In conclusion, our study confirms that ⁶⁸Ga-PSMA PET/CT is an excellent method for detecting metastatic lesions with different localisations, as well as an oligometastatic disease in the initial staging of patients with primary PC before planning radical therapy. The hybrid imaging method demonstrated a high detection rate of metastatic lesions (in 37.6% of the intermediate and high risk patients). The detection of primary PC metastases is positively related to the EAU risk group, PSA and ISUP grade values. The detection rate for metastatic lesions was significantly higher in high-risk patients (42.6%) than in intermediate-risk patients (6.6%), similar to another retrospective study. With increasing ISUP grade and PSA values, the detection rate for metastatic lesions (regional and distant LN, bone, visceral and distant metastases in total) also increases, which is consistent with the results of a retrospective study. The detection rate of possible diagnostic errors in the current study is not negligible either: false-positive and false-negative findings were found in 13.8% and 7.3% of the studied patients, respectively, which correlates with the data from the retrospective study of Fendler et al., 2018.

Good knowledge of the variations in physiological PSMA activity and possible causes of diagnostic errors is essential to optimise the interpretation of results and, therefore, for the clinical practice. The established advantages of ⁶⁸Ga-PSMA PET/CT over CT in the initial staging of primary PC before radical therapy are mainly in terms of detection of small-sized LN and bone marrow metastases (PSMA PET found additional metastatic LN in 26.6% and bone metastases in 15.6% of patients). The detection of additional metastatic lesions with the hybrid method was positively associated with the PSA levels. Our results are consistent with data from a recent prospective multicentre study by Hofman et al., 2020.

4. Influence of ⁶⁸Ga-PSMA PET/CT on regional nodal (N) and distant (M) staging compared to conventional imaging methods (CT, MRI and BSc) in patients with primary PC (group IV)

There are still ongoing discussions about the optimal imaging modality for primary staging of intermediate and high-risk PCs. The role of PSMA-PET/CT has not been sufficiently studied in this context compared to conventional imaging methods. Our study aimed to compare the hybrid imaging method ⁶⁸Ga-PSMA PET/CT with conventional imaging methods CT, MRI and BSc in assessing regional nodal N and distant metastasis (M status) in patients with histologically verified primary PC with intermediate and high risk referred for staging before radical treatment. We aimed to determine the factors related to the detection rate of regional LN and distant metastatic lesions of the conventional and hybrid method, and thus optimise the selection of patients for staging with ⁶⁸Ga-PSMA PET/CT.

Table 25. Characteristics of the conducted imaging examinations

Conducted imaging studies	n (%)
⁶⁸ Ga-PSMA PET/CT	69 (100.0)
Whole body planar BSc	69 (100.0)
SPECT/CT	28 (40.6)
MRI	48 (69.6)
Contrast-enhanced CT of the pelvis, abdomen	23 (33.3)
Contrast-enhanced CT of the pelvis, abdomen, thorax	14 (20.3)
Low-dose CT	32 (46.4)
Interval between PSMA PET/CT and conventional imaging methods, median – days (range)	46 (10-86)

BSc – bone scintigraphy, MRI – magnetic resonance imaging, CT – computed tomography

The main characteristics of the imaging studies performed in patients of group IV (n = 69) and the time range between conventional and PSMA-PET/CT scans are presented in Table 25. In all included patients, ⁶⁸Ga-PSMA PET/CT and whole-body planar BSs were performed (additional SPECT/CT of selected regions was conducted in 28 patients). Contrast-enhanced CT was performed in 37 patients, of which 23 were pelvic and abdominal CT, and 14 were pelvic, abdominal, and chest CT scans. In 32 patients, the comparative assessment was performed based on a low-dose CT scan as part of the whole-body scan protocol – Body PET/CT. Contrast-enhanced CT was not performed in 5 patients (in 2 due to allergic reactions,

in 3 – impaired renal function) and in 27 patients with PSA values below 10.0 ng/mL. MRI was also performed on 48 patients in the group. MRI was not performed in 13 patients in group IV due to the presence of metal prosthesis in 1 patient, claustrophobia in 3 patients, and noticeable distant metastatic lesions from PSMA PET/CT study in 9 patients. The test was performed in another hospital on 8 patients (and accordingly the results were not included in the present study).

Staging with conventional imaging methods

Prior to PSMA PET/CT scans, patients underwent clinical T-stage assessment with a digital rectal examination and conventional imaging studies: CT, MRI, and BSc. Experienced imaging specialists evaluated CT and MRI images according to current guidelines, including the size of the LN. CT images were evaluated for nodal and distant metastatic lesions; pelvic LN sizes > 8 mm were considered positive. Low dose CT was performed as part of the Body PET/CT whole-body scan protocol and interpreted without overlapping the metabolic PET imaging. Findings for regional nodal (N) and distant metastatic (M) staging by conventional imaging methods, including low-dose CT as well as PSMA PET/CT, were interpreted and categorised as: 1) negative for PC, 2) suspicious lesions that cannot be unequivocally defined as benign or malignant/PC, 3) positive – lesions considered as PC.

Conventional imaging methods have different scan volumes/ranges, and the examined regions did not always coincide. Therefore, they were considered as complementary modalities. The composite/aggregate data for regional nodal engagement (N status) in conventional imaging methods were obtained by grouping the results of CT and MRI imaging as follows: we considered result as a positive one in the presence of at least one positive result from conventional imaging methods, defined as dominant over negative and/or a suspicious result of the other modality; we considered a suspicious result to be dominant over the negative result of the other imaging method.

Composite data for distant metastatic involvement (M staging) derived by conventional imaging methods were obtained by grouping the results of CT, MRI, and BSc performed as follows: a positive result was considered as such in the presence of at least one positive result from conventional imaging methods, defined as dominant compared to negative and/or suspicious result of the other modalities; we considered a suspicious result to be dominant over the negative result of the other imaging methods.

Characteristics of Group IV patients

We included 8 (11.6%) intermediate-risk patients and 61 (88.4%) high-risk patients with histologically verified PC who were referred for ⁶⁸Ga-PSMA PET/CT staging before scheduling a radical treatment.

Table 26. Patients' characteristics (group IV)

Parameters	n (%)
Total number of patients	69
Age – yrs., median (range)	69 (51- 86)
PSA ng/mL, median (range)	14.42 (2.1- 1130)
Gleason score, median (range)	7 (6-10)
Gleason score	
6	7 (10.1)
7	23 (33.3)
8	20 (29.0)
9	12 (17.4)
10	7 (10.1)
EAU risk group	
intermediate risk	8 (11.6)
high risk	61 (88.4)
ADT/hormone therapy	6 (8.7)

EAU – European Association of Urology, ADT – androgen deprivation therapy during PET/CT examination

Table 26 presents the main clinical indicators characterising patients in group IV, of which 61 (88.4%) patients with high-risk PC. PSA ng/mL, median (range) reported – 14.4 (2.1-1130) ng/mL. Hormone therapy was started in 6 (8.7%) patients, and was performed during the PSMA-PET examination as well.

We distributed patients according to the ISUP grade as follows: ISUP 1 – 7 (10.1%), ISUP 2 – 14 (20.3%), ISUP 3 – 9 (13.0%), ISUP 4 – 20 (29.0%), ISUP 5 – 19 (27.5 %). More than half of the patients were with established ISUP (4-5) – 39 patients (56.5%). Patients were distributed according to the clinical T stage: cT1 – 6 (8.7%), cT2 – 40 (58.0%), cT3 – 18 (26.0%), cT4 – 5 (7.3%), with the highest percentage for cT2 stage. We divided the patients according to the PSA levels: in 27 (39.1%) patients the PSA levels were reported < 10.0 ng/mL, in 12 (17.4%) of the patients were with established PSA levels between 10.0 and 20.0 ng/mL, in 30 (43.5%) of the patients the PSA levels were > 20.0 ng/mL.

Influence of ⁶⁸Ga-PSMA PET/CT on staging

Composite staging data from the conducted conventional imaging methods were compared with the results of a staging hybrid ⁶⁸Ga-PSMA PET/CT scan – separately for nodal (N) and distant malignant (M) PC involvement. For N and M staging, the increase in stages was defined as a change from negative to suspicious or positive, as well as a change from a suspicious result to a positive one. The results of the studies ascending the stages required additional imaging and clinical confirmation in the follow-up of these patients. The reduction of the stage was considered a change from positive to suspicious or negative, as well as from suspicious to negative results. We determined the level of confidence (LoC) for the correct assessment of the PC stage based on the imaging findings separately for N and M status systematised in several categories. For both N and M staging, an increase in the confidence level was defined as a change from a suspicious to a positive or negative outcome. Decrease in the confidence level was defined as a change from positive or negative to a suspicious result.

Increased PSMA activity was found in the primary tumour of all patients. Our statistical calculations demonstrated a significant difference in the detection rate in N staging for CT, MRI and conventional N staging (from the obtained composite data) compared to ⁶⁸Ga-PSMA PET/CT, as well as in M staging for CT, BSc and conventional M staging relative to PSMA PET/CT, respectively. The results of the calculations are presented in Tables 27 and 28.

Table 27. Comparison of conventional imaging methods with ⁶⁸Ga-PSMA PET/CT in regional (N) and distant metastatic (M) staging of PC

N staging		CT, n(%)	MRI, n (%)	Conventional ¹ n (%)	⁶⁸ Ga-PSMA PET/CT, n (%)
Negative		48 (69.6)	33 (47.8)	46 (66.7)	40 (58.0)
Suspectible		7 (10.1)	9 (13.0)	9 (13.0)	0 (0.0)
Positive		14 (20.3)	6 (8.7)	14 (20.3)	29 (42.0)
M staging	BSc	CT, n(%)	MPT, n (%)	Conventional ² n (%)	⁶⁸ Ga-PSMA PET/CT, n (%)
Negative	45 (65.2)	42 (60.9)	39 (56.5)	36 (52.2)	37 (53.6)
Suspectible total	9 (13.0)	9 (13.0)	7 (10.1)	11 (16.0)	1 (1.4)
Suspectible M1a		2 (2.9)	2 (2.9)	2 (2.9)	
Suspectible M1b	9 (13.0)	6 (8.7)	5 (7.2)	8 (11.6)	1 (1.4)
Suspectible M1c		1 (1.4)		1 (1.4)	
Positive total	15 (21.7)	18 (26.1)	2 (2.9)	22 (31.9)	31 (45.0)
Positive M1a		5 (7.2)		5 (7.2)	7 (10.1)
Positive M1b	15 (21.7)	11 (15.9)	2 (2.9)	15 (21.7)	17 (24.6)
Positive M1c		2 (2.9)		2 (2.9)	7 (10.1)

BSs – bone scintigraphy, CT – computed tomography, MRI – magnetic resonance imaging, ¹ Data from conventional imaging methods by grouping CT and MRI results, ² Data from conventional imaging methods by grouping CT, MRI and BSc results

When assessing regional nodal metastasis (N status), the results of the conventional staging showed: 46 patients (66.7%) with a negative result, 9 (13.0%) with suspected results, and 14 (20.3%) with positive. Based on the additional data obtained from a hybrid ⁶⁸Ga-PSMA PET/CT study, 16 (23.2%) patients had been elevated to stage N, 5 (7.2%) patients decreased stage and 48 (69.6%) had no stage N change. The mean value, mm ± SD, when measuring the short diameter of metastatic LN with elevated N stage after PSMA PET/CT scan was calculated at 6.07 ± 1.14, median (range) 6.0 (4 – 8) mm. Table. 28 presents the results for randomly observed frequencies in N, M staging, showing a statistically significant difference in the detection rate in N and M staging between conventional (separately and from composite data) and the hybrid imaging method ⁶⁸Ga-PSMA PET/CT.

Table 28. Table for randomly observed frequencies in N, M staging

N staging				
	Negative	Suspectively	Positive	Total
Conventional*	46	9	14	69
⁶⁸ Ga-PSMA PET/CT	40		29	69
Total	86	9	43	138
X ² (2, N = 69) = 40.794; p < 0.0001				
KT	48	7	14	69
⁶⁸ Ga-PSMA PET/CT	40		29	69
Total	88	7	43	138
X ² (2, N = 69) = 35.176; p < 0.001				
MPT	33	9	6	48
⁶⁸ Ga-PSMA PET/CT	40		29	69
Total	73	9	35	117
X ² (2, N = 69) = 24.095; p < 0.001				
M staging				
	Negative	Suspectively	Positive	Total
Conventional *	36	11	22	69
⁶⁸ Ga-PSMA PET/CT	37	1	31	69
Total	73	12	53	138
X ² (2, N = 69) = 41.723; p < 0.001				
KT	42	9	18	69
⁶⁸ Ga-PSMA PET/CT	37	1	31	69
Total	79	10	49	138
X ² (2, N = 69) = 45.160; p < 0.001				
KC	45	9	15	69
⁶⁸ Ga-PSMA PET/CT	37	1	31	69
Total	82	10	46	138
X ² (2, N = 69) = 28.416; p < 0.001				

*BSc – bone scintigraphy, CT – computed tomography, MRI – magnetic resonance imaging *Data on conventional imaging methods by grouping CT, MRI and BSc results*

When assessing the distant (M) metastasis, the staging results of conventional imaging methods showed: 36 (52.2%) patients with a negative result, 11 (16.0%) – with suspicious results, and 22 (31.9%) patients with positive results. Based on the additional data obtained from a hybrid PSMA-PET/CT study, in 18 (26.1%) patients the stage was elevated, in 8 (11.6%) – decreased, and in 43 (62.3%) there was no change in M stage. The net increase of the level of confidence (LoC) in relation to M status was reported at 10 (14.5%). Calculation results (detection rate for each imaging method) are presented in Tables 27, 28. The changes made after the PSMA-PET scan – changes in N and M staging, as well as the confidence level (LoC) for the correct assessment of N and M status based on imaging studies, are presented in Table 29.

Table 29. Effect of ⁶⁸Ga-PSMA PET/CT on regional N and distant M staging

Staging	n (%)	Total Changed stage, n (%)	LoC*
N status			
Stage increase	16 (23.2)		9 (13.0%)
Stage decrease	5 (7.2)	21 (30.4)	0 (0.0%)
No change	48 (69.6)		60 (86.9%)
M status			
Stage increase	18 (26.1)		11 (15.9%)
Stage decrease	8 (11.6)	26 (37.7)	1 (1.4%)
No change	43 (62.3)		57 (82.6%)

LoC* – level of confidence for the correct assessment of N and M status based on imaging studies

Regional metastatic N involvement was detected with PSMA-PET in 29 (42.0%) patients with intermediate and high-risk PC, including 24 (34.8%) patients with PSA levels \geq 10.0 ng/mL (Table 30) and 23 (33.3%) with ISUP grade 4-5.

Distant metastatic involvement was detected with PSMA PET/CT in 31 (44.9%) patients with high-risk PC, including 24 (34.8%) patients with PSA levels \geq 10.0 ng/mL (Table 31), and 25 (36.2%) with ISUP grade 4-5.

Figure 11 shows a 66-year-old patient with histologically verified acinar adenocarcinoma of the prostate (Gleason score 8, ISUP grade 4, PSA – 41.0 ng/mL) referred for PSMA-PET staging. From the hybrid imaging study, in addition to the primary tumour formation involving both lobes of the prostate (a), we found dissemination of the disease in regional pelvic LN, as well as distant retroperitoneal retrocrural and single cervical LN to the left (a), secondary involvement of the pleura to the left (b – d) presented on the axial images. The distant metastatic LN and pleural involvement was detected only with PSMA PET/CT.

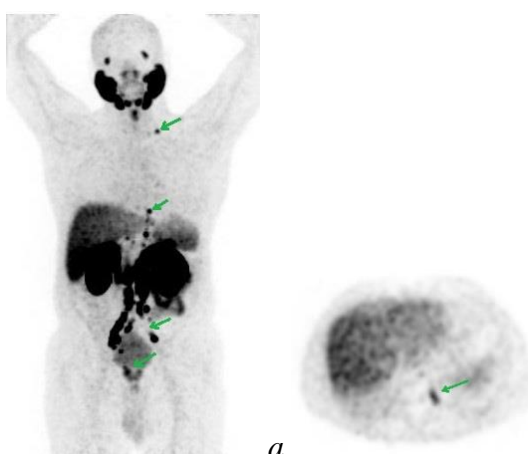


Figure 11. (a) Maximum intensity projection (MIP), (b) PET axial

Figure 12 shows a 71-year-old patient diagnosed with PC, Gleason score 8, PSA – 16.9 ng/mL. PSMA PET/CT staging detected two PSMA-expressing small metastatic LNs – presacral, pararectal (Fig. 12 a – d), with no suspicious data from low dose CT (b, d) and MRI (e). MRI imaging did not show any metastatic lesions (e).

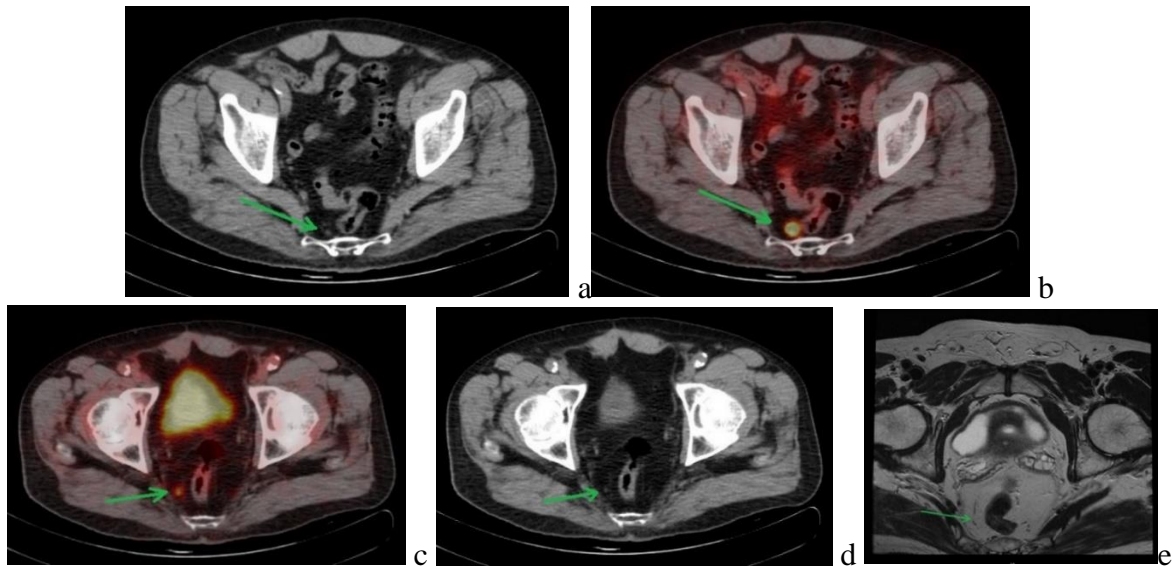


Figure 12. (a) Low dose CT, (b) fusion axial, (c) fusion axial, (d) Low dose CT, (e) MRI (AX T2)

When assessing the distribution of positive results from N staging imaging studies versus PSA values, a more significant difference between conventional methods and PSMA-PET was reported for PSA ≥ 10.0 ng/mL – 11 (26.2%) versus PSA < 10.0 ng/mL – 4 (14.8%). The lower detection rate of imaging methods was noticeable at the lower values of the tumour marker, the results are presented in Table. 30.

Table 30. Distribution of positive results from conducted conventional and ^{68}Ga -PSMA PET/CT studies in regional nodal (N) staging using a PSA limit value of 10.0 ng/mL

PSA ng/mL	Number of patients, n	CT positive, n(%)	MRI positive, n (%)	PSMA PET/CT positive, n (%)	Conventional* positive, n (%)
< 10.0	27	1 (3.7)	1 (3.7)	5 (18.5)	1 (3.7)
≥ 10.0	42	13 (31.0)	5 (12.0)	24 (57.1)	13 (31.0)
Total	69	14 (20.3)	6 (8.7)	29 (42.0)	14 (20.3)

*BSc – bone scintigraphy, CT – computed tomography, MRI – magnetic resonance imaging, *Data on conventional imaging methods by grouping CT and MRI results*

When assessing the distribution of positive results from the imaging studies for M staging compared to the PSA values, a greater difference between conventional methods and PSMA-PET was reported for PSA ≥ 10.0 ng/mL – 8 (19.0%) versus PSA < 10.0 ng/mL. The results are presented in Table. 31. In 3 out of 5 patients with a positive BSs results, the results were reported as false-positive. The detection rate for BSc was 7.4% (2 of 27 patients) with PSA levels < 10.0 ng/mL versus 25.9% for PSMA/PET.

Table 31. Distribution of positive results from conventional and ⁶⁸Ga-PSMA PET/CT studies in distant (M) staging using a PSA limit value of 10.0 ng/mL

PSA ng/mL	Number of patients, n	CT positive, n(%)	MRI positive, n (%)	CT positive, n (%)	PSMA PET/CT positive, n (%)	Conventional* positive, n (%)
< 10.0	27	2 (7.4)	0 (0.0)	5 (18.5)	7 (25.9)	6 (22.2)
≥ 10.0	42	16 (38.1)	2 (5.0)	10 (23.8)	24 (57.1)	16 (38.1)
Total	69	18 (26.1)	2 (2.9)	15 (21.7)	31 (44.9)	22 (31.9)

BSc – bone scintigraphy, CT – computed tomography, MRI – magnetic resonance imaging, *Data on conventional imaging methods by grouping CT and MRI results and CT results

When assessing the distribution of cases with changed N and M PC-stage after ⁶⁸Ga-PSMA PET/CT scan according to the PSA values, EAU risk group, clinical T stage, and Gleason score, we determined: with increasing the risk group, the frequency of changed N stage increased from 12.5% for intermediate risk to 32.8% for high risk. The same refers for M stage: the frequency of changed stage increased from 25.0% for intermediate risk to 39.3% for high-risk PC.

In conclusion, ⁶⁸Ga-PSMA PET/CT is a highly promising hybrid imaging method exceeding the conventional modalities in detecting nodal and distant metastatic lesions in the initial staging of intermediate and high-risk primary PCs prior to scheduling radical treatment. PSMA PET/CT demonstrates a higher detection rate for regional nodal (42.0%) and distant metastatic lesions (44.9%), mainly in high-risk patients with higher PSA values ≥ 10.0 ng/mL and ISUP grade 4-5. Respectively, it introduces significant changes in nodal and distant staging: when comparing ⁶⁸Ga-PSMA PET/CT with conventional imaging methods, PSMA PET/CT changed N status in 30.4% of the patients and M status – in 37.7% of patients. Our results correlate with data from other published studies. Cases with altered N and M staging are more common in patients with a high-risk EAU group, as well as in Gleason scores 8-10. False-positive BSc results reached 20.8% of BSc-positive patients (in terms of bone metastases), similar to another study. The present study results confirm the high potential of the hybrid imaging method PSMA PET/CT to replace both conventional modalities (BSs and CT) in the initial staging of high-risk patients and thus to optimise the diagnostic algorithm of primary PC, which is consistent with data from other studies.

5. ⁶⁸Ga-PSMA PET/CT in PC with ISUP grade 5 (group V)

The role of the hybrid method in patients with ISUP 5 has not yet been well investigated. Research data on the ISUP grade for PC and PSMA PET/CT are mainly aimed at determining the detection rate of the method versus the Gleason score (≤ 7 and ≥ 8), as well as the correlation between the level of PSMA expression and Gleason score and do not answer current questions about the role of the method in high-risk PC patients with ISUP grade 5. In this regard, the aim of our study was to investigate the use of ⁶⁸Ga-PSMA PET/CT in high-risk PC patients with ISUP grade 5 in three subgroups of patients: 1) patients with BCR after radical therapy; 2) patients with biochemical progression (including PSA < 0.2 ng/mL) after RP; 3) patients with primary PC referred for initial staging. The task of the present study was also to analyse the features of nodal and bone metastasis; the correlation between PSA values and the detection

rate for the different sites of PC malignant involvement in the three subgroups of patients, and to determine the relationship between clinical T stage and the detection rate for the different locations of malignant involvement in the three patient subgroups.

Characteristics of the patients in group V

The main clinical indicators characterising the patients in group V and the respective three subgroups are presented in Table 32.

Table 32. Characteristics of the patients in group V (divided into three subgroups)

Patients' parameters	Patients' parameters with BCR, n (%) Subgroup 1	Patients' parameters with BP progression, n (%) Subgroup 2	Patients' parameters referred for staging, n (%) Subgroup 3
Patients, n	16 (26.2)	22 (36.1)	23 (37.7)
Age, median – yrs. (± SD)	64.8 (7.8)	66.3 (8.3)	69.7 (7.61)
Actual PSA, median (range)	1.86 (0.2-109.4)	1.02 (0.002- 497.0)	42.4 (6.0- 193.0)
Gleason score 9	15 (93.8)	19 (86.4)	15 (65.2)
Gleason score 10	1 (6.3)	3 (13.6)	8 (34.8)
ADT/hormone therapy	11 (68.8)	10 (45.5)	2 (8.7)

BCR – biochemical recurrence, BP progression – biochemical progression after RP, ADT – androgen deprivation therapy, SD – standard deviation

We divided the patients from group V into three subgroups as follows: subgroup 1) – patients with BCR (n = 16); subgroup 2) – patients with biochemical progression (n = 22), and subgroup 3) patients referred for staging of primary PC (n = 23). ADT was performed in 11 (68.8%) of 16 patients in subgroup 1. During PSMA/PET scan, ADT was conducted in 10 (45.5%) patients in subgroup 2 and 2 (8.7%) of 23 patients in subgroup 3 (Table 32). The distribution of patients according to the values of actual PSA in the 3 subgroups is presented in Figure 13. Given the different inclusion criteria and the different indications for conducting a PSMA PET/CT scan in the three subgroups of patients, they were distributed according to the different PSA levels.

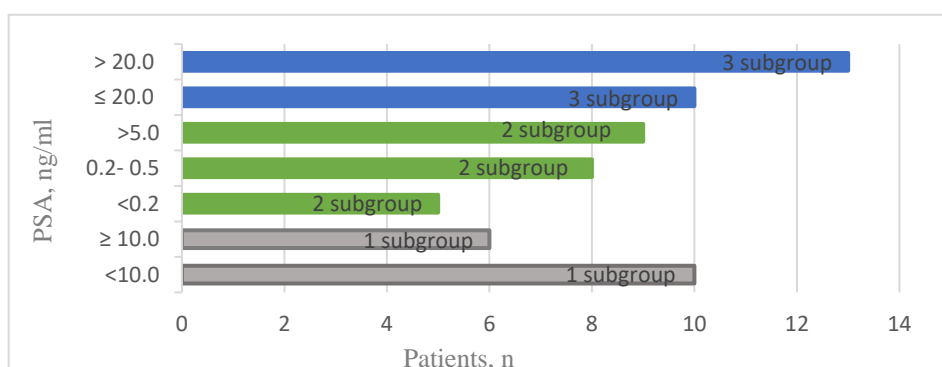


Figure 13. Distribution of patients according to the values of actual PSA (in the 3 subgroups of patients)

PSMA-PET positive and negative results in the first and second subgroup of patients were distributed as follows: for subgroup 1: 14 – positive, 2 – negative; subgroup 2: 17 – positive, 5 –negative PSMA-PET results. A higher detection rate of positive PSMA-PET results was

reported in the 1st subgroup – 87.5% compared to 77.3% in the 2nd subgroup of patients. In 17 (73.9%) of 23 patients (subgroup 3), we found metastatic lesions (regional nodal and/or distant metastases) during the staging of primary PC.

Table 33 shows the localisation of PSMA-active metastatic LNs in the ⁶⁸Ga-PSMA PET/CT scan of ISUP 5 patients in the three patient subgroups. Metastatic LNs (regional and/or distant) were found in 8 (50.0%) of 16 patients with biochemical recurrence (1st subgroup); in 10 (45.5%) of 22 patients with biochemical progression (subgroup 2), and in 11 (47.8%) of 23 patients referred for staging of primary PC (subgroup 3). Metastatic LNs (detection rate) were detected in 29 (47.5%) patients in group V (n = 61). A higher detection rate of metastatic LNs was found in patients from the 1st and 3rd subgroups (50.0% and 47.8%, respectively) compared to the 2nd subgroup (45.5%).

Table 33. Localisation of PSMA-active metastatic LNs in ⁶⁸Ga-PSMA PET/CT scan of patients with ISUP 5 (three subgroups)

Localization /regions of LN	Cases of BCR, n	Cases of BP progression, n	Staging cases, n	Total cases, n (%)
Supraphrenic (mediastinal)	2	2	3	7 (11.5)
Retroperitoneal (paraaortic, paracaval)	4	4	5	13 (21.3)
Common iliac	2	3	6	11 (18.0)
Presacral, meso para-rectal	2	2	6	10 (16.4)
Internal iliac	2	5	4	11 (18.0)
External iliac	4	4	5	13 (21.3)
Obturator	3	3	6	12 (19.7)
Inguinal, inguinal-femoral	1	3	2	6 (9.8)
Others (perivesical, mesenteric, hilus, supraclavicular)	1	1	1	3 (4.9)

BCR-biochemical recurrence, BP progression –biochemical progression, LN – lymph nodes

The localisation of PSMA-active metastatic LNs in ⁶⁸Ga-PSMA PET/CT scans of patients in group V – ISUP 5, is shown schematically in Figure 14

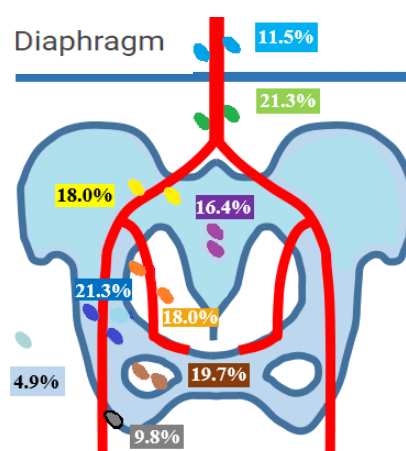


Figure 14. Localisation of PSMA-active metastatic LNs in ⁶⁸Ga-PSMA PET/CT scans of patients with ISUP 5 (group V)

The most common were the cases of detection of external iliac and retroperitoneal (para-aortic, paracaval) LN – in 21.3% of patients with ISUP grade 5 (group V), followed by obturator LN (19.7%), common iliac, internal iliac LN (18.0%), and presacral, meso para-rectal (16.4%) LN (Figure 14). A lower frequency was reported for cases with suprarenic localisation of LN (mediastinal) – 11.5%, inguinal/inguinofemoral (9.8%), and other localisations (perivesical, mesenteric, hilus, supraclavicular) in 4.9% of patients. False-negative PSMA-inactive metastatic LN were found in 3 (4.9%) patients in group V (confirmed by follow-up).

PSMA-PET study found pelvic LN with increased size but without increased PSMA-activity. After pelvic lymph node dissection was performed, the pelvic LN were histologically verified as metastatic PC lymph nodes (1 patient with BCR from the 1st subgroup, 1 patient with BP progression from the 2nd and 1 patient from the 3rd subgroup). The distribution of bone metastases according to the type of lesions in the three subgroups of patients with ISUP 5 is presented in Table 34 and on Figure 15. In 9 (56.3%) of 16 patients in the 1st subgroup, we found bone metastases, in the 2nd subgroup – in 14 (63.6%) of 22 patients, and in the 3rd subgroup we found metastatic bone lesions in 11 (47.8%) of 23 patients. Metastatic bone involvement was found in 34 (55.7%) of 61 patients (group V), of which sclerotic PSMA-active bone lesions were most often detected (34.4%) – in 21 patients. Single lesions (up to 5 in number) were found in 13 (21.3%) patients, followed by single (up to 5 in number) bone marrow lesions (18.0%) – in 11 patients.

Table 34. Characteristics of metastatic bone lesions detected in ⁶⁸Ga-PSMA PET/CT scans, cases of detection by type of lesions (group V)

BCR cases (subgroup 1)			
Bone lesions	PSMA-active single, n	PSMA-active multiple, n	PSMA - inactive, n
sclerotic	3	2	2
osteolytic	2		1
bone marrow	3		
mixed type	2		
Cases of BP progression (subgroup 2)			
Bone lesions	PSMA-active single, n	PSMA-active multiple, n	PSMA - inactive, n
sclerotic	6	3	2
osteolytic	1		
bone marrow	4		
mixed type	3		
Staging cases (subgroup 3)			
Bone lesions	PSMA-active single, n	PSMA-active multiple, n	PSMA - inactive, n
sclerotic	4	3	1
osteolytic	1	1	1
bone marrow	4		
mixed type	3		
Total cases (group V)			
Bone lesions	PSMA-active single, n	PSMA-active multiple, n	PSMA - inactive, n
sclerotic	13 (21.3)	8 (13.1)	5 (8.2)
osteolytic	4 (6.6)	1 (1.6)	2 (3.3)
bone marrow	11 (18.0)		
mixed type	8 (13.1)		

BCR – biochemical recurrence, BP – biochemical progression

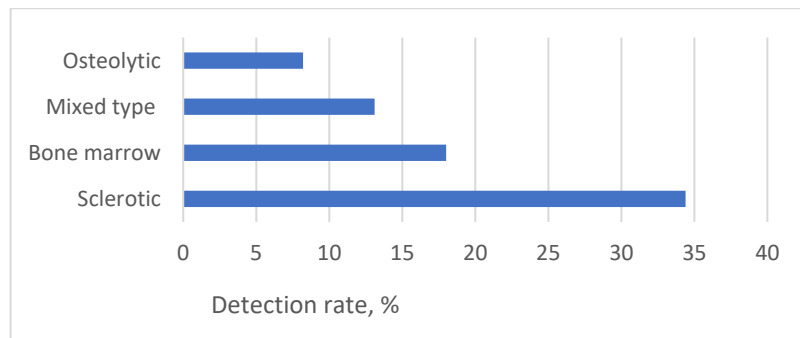


Figure 15. Detection rate of PSMA-active bone metastatic lesions according to the type of lesion in ^{68}Ga -PSMA PET/CT scan of patients with ISUP grade 5 (group V)

Multiple PSMA-active sclerotic bone metastases were found in 8 (13.1%) patients and multiple osteolytic lesions – in only one (1.6%) patient out of a total of 5 (8.2%) patients with established osteolytic lesions (Fig. 15). False-negative bone findings were found in 7 (11.5%) patients: PSMA-inactive sclerotic metastatic lesions (confirmed by follow-up) were found in 5 (8.2%) patients, as well as osteolytic lesions – in 2 (3.3%). At least one PSMA-active secondary bone lesion was detected in all of these patients.

The distribution of the pathological ^{68}Ga -PSMA PET/CT results (detection rate) according to the localisation of the PC malignant involvement in the three subgroups of patients is presented in Table. 35. A higher detection rate of distant metastatic lesions (total) was reported for patients in the 1st and 2XA subgroups (77.3% and 75.0%, respectively) compared to the patients with primary PC referred for staging (60.9%). A lower frequency of regional and distant metastatic LN detection was reported for the 2nd subgroup (27.3%) compared to the other subgroups. The detection rate and local recurrence was lower than in the 1st subgroup (9.1% compared to 31.3%, respectively). When assessing bone metastases, we found a high detection rate in all subgroups, the highest being in the 2nd subgroup (63.6%). The highest detection rate was found for distant metastases in all subgroups of patients and in the assessment of group V (70.5%).

Table 35. Distribution of positive ^{68}Ga -PSMA PET/CT results according to the localisation of PC malignant involvement in the three subgroups

Patients	Patients, n	Local recurrence, n (%)	Regional LN, n(%)	Distant LN, n(%)	Bone, n (%)	Visceral, n (%)	Distant total, n (%)	PET/CT positive, n (%)
Subgroup 1	16	5 (31.3)	8 (50.0)	7 (43.8)	9 (56.3)	1 (6.3)	12 (75.0)	14 (87.5)
Subgroup 2	22	2 (9.1)	6 (27.3)	6 (27.3)	14 (63.6)	2 (9.1)	17 (77.3)	17 (77.3)
Subgroup 3	23	-	11 (47.8)	6 (26.1)	11 (47.8)	3 (13.0)	14 (60.9)	-
Group V	61	-	25 (41.0)	19 (31.1)	34 (55.7)	6 (9.8)	43 (70.5)	-

The distribution of the positive PSMA-PET results (detection rate) at the different localisations of PC metastatic lesions according to the PSA values (a subgroup of patients with BCR) is presented in Table. 36. We did not find a statistically significant correlation between the detection rate of metastatic lesions and the PSA values. A correlation tendency was reported for the regional, distant LN and distant metastases in total. The frequency of PSMA- PET/CT positive results (detection rate in total) was higher in PSA ≥ 10.0 ng/mL (100.0%) compared to PSA < 10.0 ng/mL (80.0%).

Table 36. Distribution of positive/pathological ⁶⁸Ga-PSMA PET/CT results at different localisations of metastatic PC lesions and local recurrence according to the PSA values (subgroup 1, patients with BCR)

Actual PSA, ng/mL	Patients, n	Local recurrence, n (%)	Regional LN, n(%)	Distant LN, n(%)	Bone, n (%)	Visceral, n (%)	Distant total, n (%)	PET/CT positive, n (%)
< 10.0	10	3 (30.0)	4 (40.0)	3 (30.0)	7 (70.0)	0 (0.0)	7 (70.0)	8 (80.0)
≥ 10.0	6	2 (33.3)	4 (66.6)	4 (66.6)	2 (33.3)	1 (16.7)	5 (83.3)	6 (100.0)
p value		0.889	0.954	0.869	0.152	0.182	0.254	0.242

The detection rate of detection of metastatic bone lesions was reported higher (70.0%) at lower PSA values < 10.0 ng/mL compared to 33.3% at PSA ≥ 10.0 ng/mL (no statistically significant correlation found) (Table 36), unlike other malignant involvement sites of PC.

The distribution of positive ⁶⁸Ga-PSMA PET/CT results (detection rate) at different localisations of metastatic PC lesions and local recurrence according to the PSA values in patients with BP progression (subgroup 2) is presented in Table. 37. We found a statistically significant correlation between the detection rate of metastatic lesions and the PSA values for distant LN (p = 0.041), distant metastases in total (p = 0.002), and the overall detection rate (positive PSMA-PET results), p = 0.002. The detection rate for distant metastases increased from 20.0% (for PSA values < 0.2 ng/mL) to 100.0% for PSA > 5.0 ng/mL, and the overall detection rate increased from 20.0% to 100.0% for the respective PSA levels. However, in patients with PSA < 0.2 ng/mL we did not find distant LN – 0.0%, detection rate – 55.6% in PSA > 5.0 ng/mL.

Table 37. Distribution of positive/pathological ⁶⁸Ga-PSMA PET/CT results in local recurrence and different localisations of metastatic PC lesions according to the PSA values (subgroup 2, patients with BP progression)

Actual PSA, ng/mL	Patients, n	Local recurrence, n (%)	Regional LN, n(%)	Distant LN, n(%)	Bone, n (%)	Visceral, n (%)	Distant total, n (%)	PET/CT positive, n (%)
<0.2	5	0 (0.0)	0 (0.0)	0 (0.0)	1 (20.0)	0 (0.0)	1 (20.0)	1 (20.0)
0.2- 5.0	8	0 (0.0)	2 (25.0)	1 (12.5)	6 (75.0)	0 (0.0)	7 (87.5)	7 (87.5)
>5.0	9	2 (22.2)	4 (44.4)	5 (55.6)	7 (77.8)	2 (22.2)	9 (100.0)	9 (100.0)
p value		0.204	0.199	0.041	0.069	0.204	0.002	0.002

In patients with PSA < 0.2 ng/mL we found no local recurrence, regional and distant metastatic LN, and no visceral metastases. The detection rate for bone metastases and distant metastases was low – 20.0%. The detection rate for regional metastatic LN increased from 25.0% for PSA values (0.2 – 0.0 ng/mL) to 44.4% for PSA > 5.0 ng/mL.

The distribution of positive ⁶⁸Ga-PSMA PET/CT results (detection rate) at different localisations of metastatic PC lesions according to the PSA values in patients referred for primary PC staging (subgroup 3) is presented in Table. 38. We found a statistically significant correlation between the detection rate of distant metastatic LN and the PSA values (p = 0.012). We found a similar correlation tendency for other localisations (regional LN, bone, visceral metastases and distant metastases in total).

Table 38. Distribution of positive ^{68}Ga -PSMA PET/CT scores at different sites of metastatic PC lesions according to PSA values (subgroup 3, patients targeted for primary PC staging)

Actual PSA, ng/mL	Patients, n	Local recurrence, n (%)	Regional LN, n(%)	Distant LN, n(%)	Bone, n (%)	Visceral, n (%)
≤ 20.0 ng/mL	10	3 (30.0)	0 (0.0)	4 (40.0)	0 (0.0)	4 (40.0)
> 20.0 ng/mL	13	8 (61.5)	6 (46.2)	7 (53.8)	3 (23.1)	10 (76.9)
p value		0.133	0.012	0.510	0.292	0.072

The detection rate of regional metastatic LN increased from 30.0% for PSA values ≤ 20.0 ng/mL to 61.5% for PSA > 20.0 ng/mL. The detection rate of distant metastases increased from 40.0% for PSA values ≤ 20.0 ng/mL to 76.9% for PSA > 20.0 ng/mL.

Figure 16 presents a 74-year-old patient with a diagnosed PC with Gleason score 5 + 5 = 10, ISUP grade 5, (cT4 – bladder involvement), PSA – 28.0 ng/mL. Staging PSMA PET/CT scan detected metastatic bone involvement (a) as well as a solitary secondary lesion in the 4th segment of the liver, presented on the axial fusion image (b), detected only by the hybrid imaging method.

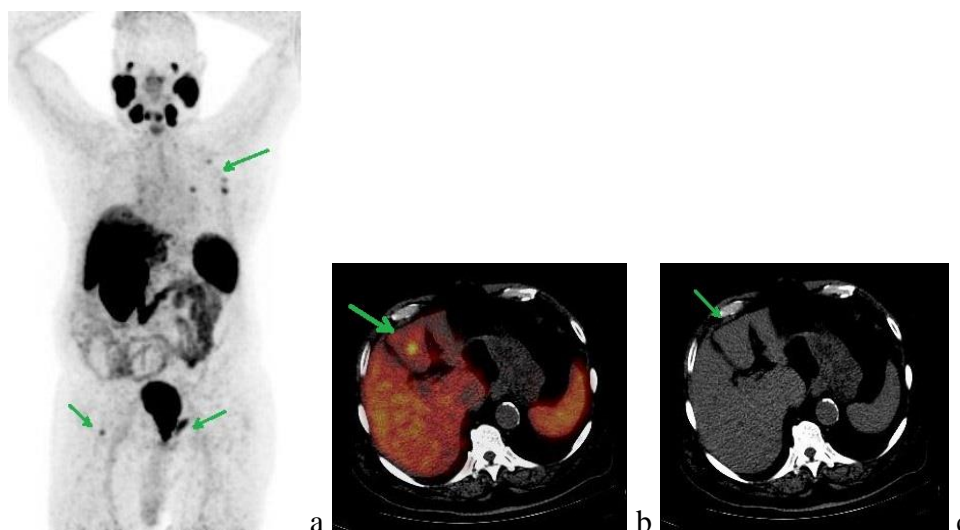


Figure 16. (a) Maximum intensity projection (MIP) (b, c) fusion, low dose CT axial

When assessing the distribution of pathological ^{68}Ga -PSMA PET/CT results in different localisations of metastatic lesions and local recurrence of PC according to the clinical T stage in patients with BCR (subgroup 1) and patients referred for staging (subgroup 3), we did not establish a statistically significant correlation between the detection rate of metastatic lesions and the clinical T stage ($p > 0.05$). A correlation tendency was found for distant LN and distant metastases in total: the detection rate increased with increasing the clinical T stage.

Our results showed a significantly higher detection rate for group V, and the three subgroups of patients compared to the first three groups studied (I – III).

In conclusion, ^{68}Ga -PSMA PET/CT demonstrates high potential in assessing patients with ISUP grade 5. The present study revealed a higher detection rate of recurrent PC in patients with BCR (87.5%) compared to patients with BP progression after RP (77.3%). The detection rate of metastatic lesions is high (73.9%) in patients with primary PC referred to staging.

Metastatic involvement of PC in patients with ISUP 5 (group V) is mainly due to the distant metastases in total: the highest in the detection rate for distant metastases (total) in the assessment of the whole group (70.5%), as well as for the three separate subgroups of patients. The detection rate is high for metastatic LN (regional and/or distant) (47.5%), the most common are the external iliac and retroperitoneal LN (21.3%) cases. The detection rate for bone metastases is high (55.7%), the most common are the PSMA-active sclerotic bone lesions (34.4%) cases. The frequency is also relatively high for false-negative bone findings (11.5%). In patients with BP progression after RP, there is a statistically significant positive correlation between the PSA values and the detection rate of distant LN ($p = 0.041$), distant metastases in total ($p = 0.002$) and the overall detection rate (positive PSMA-PET results), $p = 0.002$. In patients with primary PC referred for staging, there is a statistically significant positive correlation between the detection rate for distant metastatic LN and the PSA values ($p = 0.012$). There is no significant correlation between the detection rate of metastatic lesions and the clinical T stage ($p > 0.05$). Our results are consistent with the data from other published studies.

6. ^{68}Ga -PSMA PET/CT parameters in the studied diagnostic groups of patients (I, II, III and V)

The studies assessing the method's parameters focus mainly on the comparative evaluation of the hybrid ^{68}Ga -PSMA PET/CT method with conventional modalities (CT, MRI, BSc, and choline PET) in patients with BCR. However, the parameters of PSMA-PET for staging primary PC have not yet been well investigated. In the researched literature, we did not find enough data on the method's parameters in patients with low-grade PC with ISUP 5. So far in Bulgaria, the method's parameters have not been evaluated in a large cohort of patients. The task of our study was to determine the parameters of PSMA-PET: detection rate, sensitivity, specificity, PPV, NPV and accuracy for the different diagnostic groups of patients (with biochemical recurrence – group I, with biochemical progression after RP including values of PSA < 0.2 ng/mL – group II, with primary PC – group III, and ISUP grade 5 – V group).

The main clinical indicators characterising the patients in groups I, II, III and V are presented in the section Results and Discussion for the respective sets of tasks (groups of patients).

Determining the detection rate with PSMA PET/CT in the studied groups of patients

A higher detection rate of pathological results (for recurrent PC) was found in patients with BCR after radical therapy compared to patients with biochemical progression after RP: 67.7% compared to 43.1%. We allocated PSMA-PET positive and negative results in patients from groups I and II: for group I: 90 (67.7%) were positive, 43 – negative; for group II – 62 (43.1%) were positive, 82 – negative. When evaluating patients with primary PC (group III), the detection rate for distant metastatic lesions was slightly higher (M – 28.4%) compared to regional nodal metastases (N – 26.6%). A high detection rate was reported for metastatic lesions in total (N and/or M) – 37.6%.

The detection rate for group V (in the three patients' subgroups) was significantly higher (87.5%, 77.3% – for recurrent PC in subgroups 1 and 2, respectively, and 73.9% – for metastatic lesions of primary PC) compared to the first three groups of patients (I, II and III). We found a

higher detection rate for patients with BCR – 87.5% (subgroup 1) than in the other two subgroups.

Validation of patients with BCR after radical therapy (group I)

Histopathologically, 15 (16.6%) of 90 patients with pathological PSMA-PET results were monitored and verified. Re-biopsy due to local recurrence was performed in 6 patients, lymph dissection – in 8 patients, and biopsy of a single lung nodule – in 1 patient. In 14 of 15 patients, the results were confirmed to be true positive. In only one patient (patient-based assessment) with suspected metastatic PSMA-expressing lung nodule, metachronous primary non-small cell lung cancer (false positive) was confirmed. In the remaining 75 of 90 patients, PSMA-positive findings (bone involvement – 37 of 43 with local recurrence and 29 of 37 with nodal metastases) were validated by the follow-up (49 patients) with at least one of the imaging methods – PSMA-PET, CT, MRI or planar BSc/SPECT/CT and clinical follow-up. In all these patients, the results were confirmed to be true positive. In one out of 43 patients with a negative ⁶⁸Ga-PSMA PET/CT scan (with increased pelvic LN from CT monitoring of the abdomen and pelvis), lymph node dissection was performed, and PC was histologically verified with neuroendocrine differentiation (false negative).

In the remaining 42 patients with PSMA-PET negative, the results of conducted conventional imaging methods were negative (true negative). No progression from imaging or clinical follow-up to the end of the follow-up period was found in any of these patients. In validating suspected false-negative findings (lesion level assessment) in 2 patients (ISUP grade 5 with detected pelvic LN with increased size but without increased PSMA activity from PET/CT examination in the follow-up and after pelvic lymph dissection), they were histologically verified as metastatic LN from PC. In 3 patients with ISUP 5, PSMA-inactive metastatic bone lesions were found in a follow-up: in 2 patients – single sclerotic bone lesions, in 1 – an osteolytic lesion. Sclerotic lesions were monitored by BSc and MRI, and the osteolytic lesion – by MRI. The follow-up period was median/range of 6 months (mean 6 months/1–12 months).

Validation of patients with biochemical progression after RP (group II)

In the follow-up of patients after restaging ⁶⁸Ga-PSMA PET/CT, 9 (14.5%) of 62 patients with a positive PSMA-PET result were histopathologically verified, and re-biopsy due to local recurrence was performed in 4 (6.5%) patients; lymph node dissection was performed in 5 (8.1%). The results were confirmed as true positive (malignant PC involvement) in all these patients. In the remaining 53 (85.5%) patients, the PSMA-positive findings were validated by a follow-up with at least one of the imaging methods – PSMA-PET, CT, MRI and/or scintigraphic (planar BSc/SPECT/CT), as well as a clinical follow-up. PSMA PET/CT results were confirmed as true positive in all these patients. A PSMA-PET negative result was reported in 82 (56.9%) of 144 patients. In one of these patients (with ISUP 5 and PSA level 0.32 ng/mL) during PET/CT scan, a contrast-enhanced CT scan of the abdomen and pelvis revealed increased pelvic LN. After pelvic lymph node dissection, they were histologically confirmed as PC metastatic lymph nodes (false-negative result). In the remaining negative PSMA-PET patients, the results of the conducted correlative conventional imaging methods were negative (true negative), and there was no evidence of progression from imaging or clinical follow-up. The follow-up period was median/range of 6 months (mean 6 months/1–12 months).

Validation of patients with primary PC (group III)

In the follow-up of patients in group III after staging, 28 (25.7%) patients underwent conventional RP, 13 (11.9%) – robot-assisted RP, 1 (0.9%) patient – HIFU, and laser vaporization – in 2 (1.8%) patients. In 35 of these patients (n = 44), low PSA values < 0.01 ng/mL were reported after treatment (excluding hormone therapy), confirming the absence of distant metastatic lesions, as established by a staging PSMA PET/CT scan test. In 9 patients, 8 of whom were interpreted as M negative, 1 – suspicious (M1b) from the PSMA-PET scan, postoperative biochemical remission was not reported. The biochemical persistence was probably due to regional nodal malignant involvement, with metastatic regional LN histologically confirmed in 8 of 9 patients after pelvic lymph node dissection. A follow-up restaging PSMA-PET was performed within 1 year after surgery (median – 6 months, range 3 – 12 months) in the 9 patients with biochemical persistence: in 4 out of 8 (with histologically verified regional metastatic LN) patients, nodal metastatic lesions were detected, as the only localisation of recurrent PC; multiple localisations including metastatic LN were found in 2 patients, and negative results – in 3.

All 9 (8.3%) patients with metastatic regional LN (positive results) without distant metastatic lesions (PSMA-PET scan) were histopathologically verified after pelvic lymph node dissection. Validation by a follow-up with at least one of the imaging methods – PSMA-PET, CT, MRI or BSc, as well as a clinical follow-up was performed in the following: patients with detected distant metastases (positive PSMA-PET results – 31 (28.4%), of which 8 (7.3%) patients – with oligometastatic involvement (≤ 5 lesions); 23 (21.1%) patients with bone involvement, of which in 8 (7.3%) disseminated bone involvement; 14 (12.8%) – with distant metastatic LN, all of which had regional metastatic LN; 4 (3.7%) – with visceral metastatic involvement from PC. The results of all these patients were confirmed to be true positive for PSMA-PET. The follow-up period was median/range of 6 months (mean 6 months/3 – 12 months). In one (0.9%) patient, the histologically verified primary PC (GS 9, ISUP grade 5), the PSMA antigen was not expressed. No other findings with increased RP activity (false-negative result) were detected in the same patient, despite the significantly elevated PSA values – 41.46 ng/mL.

Validation of suspicious false-positive and/or negative findings (group III)

In all 3 cases of false-negative findings for metastatic LN, lung and liver metastases were histologically verified in the follow-up. In contrast, false-negative findings for bone metastases in 4 patients were confirmed by the follow-up with two imaging modalities: in 2 patients with sclerotic lesions – with BSc and MRI, in 2 patients with osteolytic lesions – with CT and MRI. Some of the false-positive follow-up findings were histologically verified: benign thyroid nodule (follicular adenoma) – found in two patients, synchronous primary neoplastic processes (sigmoid colorectal adenocarcinoma – in one patient, squamous cell lung cancer – in 1 patient, lung adenocarcinoma – in 2 patients). Other false-positive findings such as bone fractures, Paget's disease, vertebral and visceral hemangiomas (hepatic and lienal), celiac ganglion, inflammatory paraaortic LN were confirmed by follow-up with CT and/or MRI imaging methods, ultrasound (for lienal hemangiomas), and PSMA PET/CT for the patient with suspicious inflammatory paraaortic LN.

Validation of patients (group III) with PSMA-PET negative (false-negative) results during evaluation for metastatic lesions

In two of the monitored patients without PSMA-PET verified metastatic lesions, the presence of the latter was confirmed (false-negative results for metastases): in one patient – metastatic regional LN (histological verification after pelvic lymph node dissection); in the second patient – osteolytic bone metastases (confirmed by MRI and CT). ISUP 5 (Gleason score 9 and 10, respectively) was reported in both patients.

Validation of patients (group V, subgroup 1)

In the follow-up of patients in subgroup 1 (n = 16) after restaging ⁶⁸Ga-PSMA PET/CT, 4 (25.0%) of 16 patients in the subgroup were histopathologically verified. Re-biopsy due to local recurrence was performed in 1 (6.3%) patient, lymphatic dissection – in 3 (18.8%) patients. The results were confirmed to be true positive (malignant involvement from PC) in all these patients. For the remaining 12 (75%) of 16 patients, the PSMA-positive findings (bone involvement in 9 (56.3%) patients, as well as 4 of 5 patients with local recurrence, and 5 of the 8 – with nodal metastases) were validated by follow-up with at least one of the imaging methods – PSMA-PET, CT, MRI and/or scintigraphic (planar BSc/SPECT/CT), as well as clinical follow-up. In all these patients, PSMA-PET results were confirmed to be true positive. In 2 (12.5%) of 16 patients, PSMA-PET was reported as negative.

In one of these patients, with a negative PSMA-PET scan and PSA levels of 0.52 ng/mL during the test, but increased pelvic LN from a restaging CT examination of the abdomen and pelvis (performed 1.5 months after PSMA-PET), in the follow-up – a pelvic lymph node dissection was performed, and metastatic LN from PC with neuroendocrine differentiation (false-negative result) were histologically verified. In the other patient with PSMA-PET negative, the results of conventional imaging methods were negative (true negative), and no progression from imaging or clinical follow-up to the end of the follow-up period was detected. The follow-up period of patients in the subgroup (median/range) was 6 months (6 months /1 – 12 months).

Patient validation (group V, subgroup 2)

When monitoring patients from subgroup 2 (n = 22) after restaging ⁶⁸Ga-PSMA PET/CT, 3 (13.6%) of 22 patients in the subgroup were histopathologically verified; re-biopsy due to local recurrence was performed in 1 (4.5%) patient, lymphatic dissection – in 2 (9.1%) patients. The results were confirmed to be true positive (malignant involvement of PC) in all these patients. In the remaining 19 (86.4%) patients, the PSMA-positive findings (bone involvement in 14 (63.6%) patients, as well as the second patient with local recurrence and 8 out of 10 with nodal metastases) were validated by follow-up with at least one from the imaging methods – PSMA-PET/CT, CT, MRI and/or BSc (planar/SPECT/CT), and a clinical follow-up.

PSMA PET/CT results were confirmed as true positive in all these patients. A PSMA-PET negative result was reported in 5 (22.7%) of 22 patients. In one of these patients with a PSA level of 0.32 ng/mL during PET/CT scan, a contrast-enhanced CT scan of the abdomen and pelvis revealed increased pelvic LN. Metastatic LN from PC (false-negative result) were histologically confirmed after pelvic lymph node dissection. The results of the conventional imaging methods for the remaining patients with negative PSMA-PET were negative (true negative), and there was no evidence of progression from imaging or clinical follow-up. The

follow-up period of patients in the subgroup (median/range) was 6 months (6 months /1 – 12 months).

Validation of patients (group V, subgroup 3)

In the follow-up of patients in subgroup 3 (n = 23) after PSMA-PET staging, 6 (26.1%) patients (no regional nodal and/or distant metastases detected) underwent conventional RP. In 5 (21.7%) of these patients, low PSA values < 0.01 ng/mL were reported after surgery, confirming the absence of distant metastases, as found in a staging PSMA-PET examination. Postoperative biochemical remission was not reported in 1 (4.3%) patient. The biochemical persistence was due to regional nodal malignant involvement histologically confirmed after pelvic lymph node dissection. 3 (13.0%) patients with detected metastatic regional LN-positive results (without distant metastatic lesions verified by PSMA-PET) were histopathologically monitored and verified after pelvic lymph node dissection.

Validation by at least one of the imaging methods – ⁶⁸Ga-PSMA PET/CT, CT, MRI and/or BSc, as well as a clinical follow-up, was conducted for the patients with detected distant metastases (positive PSMA-PET results – 14 (60.9%), of which 3 (13.0%) patients – with oligometastatic involvement (≤ 5 lesions), 11 (47.8%) patients with bone involvement, 4 (17.4%) – with disseminated bone involvement, 6 (26.1%) – with distant metastatic LN, all of which had regional metastatic LN, 3 (13.0%) – with visceral metastatic LN involvement from PC. The results of all these patients were confirmed to be PSMA-PET true positive. In one (4.3%) patient with histologically verified primary PC (GS 9, ISUP grade 5), there was no expression of PSMA antigen. Despite the increased PSA values of up to 41.46 ng/mL in this patient, we also did not detect other findings with increased RP activity (false-negative result). In the follow-up of the same patient, regional metastatic LN (not expressing PSMA from the staging PET/CT) was found. The follow-up period of patients in the subgroup (median/range) was 6 months (6 months /3 – 12 months).

Validation of patients (group V, subgroup 3) with PSMA-PET negative (false-negative) results when evaluating for metastatic lesions

In two of the monitored patients without metastatic lesions identified by PSMA-PET, the presence of the latter was confirmed (false-negative results for metastases): in one patient – metastatic regional LN (histological verification after pelvic lymph node dissection); in the second patient – osteolytic bone metastases (confirmed by MRI and CT). ISUP 5 (Gleason score 9 and 10, respectively) was reported in both patients. The follow-up period of patients in the subgroup (median/range) was 6 months (6 months /3 – 12 months).

Validation of suspicious false-negative findings (lesion level assessment), group V

In one (1.6%) of 61 patients with ISUP 5, the histologically verified primary PC (Gleason score 9, ISUP grade 5) did not express PSMA antigen (subgroup 3). In the follow-up of 3 (4.9%) patients of group V with detected pelvic LN with increased size but without increased PSMA-activity from PET/CT examination, metastatic LN from PC (one patient from each subgroup) were histologically verified after pelvic lymph dissection. In 7 (30.4%) patients with false-negative PSMA-inactive findings for bone metastases, the latter was confirmed by follow-up with two imaging methods: in 5 (21.7%) patients with sclerotic lesions – with BSc and MRI, in 2 (8.7%) patients with osteolytic lesions – with CT and MRI. A metastatic PC lesion was

histologically verified after biopsy in 1 patient (subgroup 3) with a detected lung nodule without increased PSMA activity from PET/CT.

Evaluation of the ^{68}Ga -PSMA PET/CT parameters: sensitivity, specificity, PPV, NPV and accuracy

The distribution of true positive/negative PSMA-PET results (TP/TN), as well as false positive/negative PSMA-PET results (FP/FN) in the studied groups of patients, are presented in Table 39. The assessment was performed on a patient-by-patient basis. In patients of group III and subgroup 3 (group V), respectively, the metastatic lesions (regional nodal and/or distant metastases) were assessed. Metastatic lesions were found in 41 patients (group III). In 2 patients, we found false-negative results regarding metastatic lesions: in one – metastatic regional LN, in the second patient – osteolytic bone metastases. When evaluating patients in group V (n = 61), recurrent PC was found in 31 of 38 patients with BCR and biochemical progression after RP (subgroups 1 and 2), metastatic lesions were found in 17 of 23 patients (subgroup 3): a total of 48 PSMA-PET positive results.

Table 39. Distribution of true positive/negative PSMA-PET results (TP/TN), as well as false positive/negative PSMA-PET results (FP/FN) in patients of groups I, II, III and V

	Patients, n	PSMA-PET positive	PSMA-PET negative	TP	TN	FP	FN
Group I	133	90	43	89	42	1	1
Group II	144	62	82	62	81	0	1
Group III	109	41	68	41	66	0	2
Group V	61	48	13	48	9	0	4

TP – true positive, TN – true negative, FP – false positive, FN – false negative

The main parameters (sensitivity, specificity, PPV, NPV, and accuracy) of the hybrid imaging method in the studied diagnostic groups of patients (groups I, II, III and V) are presented in Table 40. In all studied diagnostic groups of patients, we reported high specificity (from 97.7% to 100.0%) and PPV (from 98.9% up to 100.0%), given that only one patient (from group I) was found in follow-up with a false-positive result – metachronous primary non-small cell lung cancer.

Table 40. Main ^{68}Ga -PSMA PET/CT parameters in the diagnostic groups of patients (with BCR – group I, with BP progression after RP – group II, with primary PC – group III, and with ISUP 5 – group V)

	Sensitivity,%	Specificity,%	PPV,%	NPV,%	Accuracy,%
Group I	98.9	97.7	98.9	97.7	98.5
Group II	98.4	100.0	100.0	98.8	99.3
Group III	95.3	100.0	100.0	97.1	98.2
Group V	92.3	100.0	100.0	69.2	93.4

PPV – positive predictive value, NPV – negative predictive value

When assessing the sensitivity of the hybrid imaging method, high values were reported in all diagnostic groups of patients, with relatively lower sensitivity (92.3%) reported for group V (patients with ISUP grade 5) compared to the first three groups (from 95.3% to 98.9 %). When assessing the negative predictive value of PSMA PET/CT, lower values were reported in

patients with ISUP grade 5/group V (69.2%) compared to the first three groups of patients (from 97.1% to 98.8%). The accuracy of the hybrid imaging method in all groups of patients was reported to be high: from 93.4% to 99.3%. We calculated relatively lower values for patients with ISUP grade 5, group V (93.4%) than the first three groups (98.2% – 99.3%).

In conclusion, we should emphasise that ⁶⁸Ga-PSMA PET/CT is a promising imaging modality with notable indicators for PC detection in BCR after radical therapy, biochemical progression after RP, and metastatic lesions in primary PC staging, especially in well-differentiated PC. The present study demonstrated high indicators for the parameters of the hybrid imaging method: detection rate, sensitivity (from 92.3% to 98.9%), specificity (97.7% – 100.0%), PPV (98.9% – 100.0%), NPV (69.2% – 98.8 %) and accuracy (93.4% – 98.5%) in all groups of patients studied: with BCR after radical therapy (group I), with biochemical progression after RP (including PSA < 0.2 ng/mL) (group II), in patients referred for staging (group III), as well as with ISUP grade 5 patients (group V).

Our results are consistent with data from other published studies. The highest detection rate was found in patients with ISUP grade 5: for subgroup 1 (87.5%), subgroup 2 (77.3%), subgroup 3 (73.9%) compared to the diagnostic groups of patients (I – III). However, the other ⁶⁸Ga-PSMA PET/CT parameters in patients with ISUP grade 5 (group V): NPV (69.2%), sensitivity (92.3%) and accuracy (93.4%) showed lower results compared to the first three groups, i.e. NPS (97.1% – 98.8%), sensitivity (95.3% – 98.9%), accuracy (98.2% – 99.3%). At the same time, the specificity and PPV for group V are very high – 100.0%. Early detection of PC recurrences (essential at low serum PSA levels), as well as the detection of metastatic lesions in PC staging (intermediate and high risk) with diagnostic modality with high sensitivity, specificity, PPV, NPV and accuracy such as ⁶⁸Ga-PSMA PET/CT, is critical and clinically important for the additional screening of patients in determining the subsequent therapeutic approach.

V. CONCLUSIONS AND RECOMMENDATIONS FOR THE CLINICAL PRACTICE BASED ON OUR EXPERIENCE

1. Conclusions

1. **⁶⁸Ga-PSMA PET/CT is an imaging method with outstanding potential and a high detection rate for recurrent PC in patients with BCR after radical therapy**, even with low PSA values during the examination (45% for PSA < 0.5 ng/mL). The detection rate is positively correlated to the initial and actual PSA levels. Actual PSA values constitute a significant predictor of PSMA-PET positive/pathological outcomes.
2. **⁶⁸Ga-PSMA PET/CT significantly exceeds conventional CT in detecting recurrent PC after radical therapy**: 86.0% local recurrence was detected only by the hybrid imaging method, 54.0% metastatic LN, 46.5% bone metastases. Additional PSMA-active lesions were found in 40% of patients with PSMA-PET-positive results.
3. **PSMA-PET is an excellent imaging method for detecting recurrent PC in patients with biochemical progression after RP** with a relatively high detection rate (15.8%) and PSA-based sensitivity (15.0%) even at very low PSA levels (> 0.04 - 0.16 ng/mL) – below the definition of BCR, which is essential for the clinical determination of subsequent therapeutic approach. The present study results suggest a future change in the definition of BCR to lower PSA values. Biochemical progression after RP is more commonly associated with distant metastatic lesions (32.6%), especially with bone metastases in patients with lower PSA levels, even below 0.04 ng/mL. The detection of recurrent PC after RP is positively associated with PSA values and Gleason scores. The detection rate for distant metastases is associated with a higher Gleason score: 61.1% for Gleason score (8-10) versus 23.1% for Gleason score (6-7).
4. **⁶⁸Ga-PSMA PET/CT is the method of choice for the detection of metastatic lesions with different localisations, including oligometastatic disease, in the initial staging of patients with primary PC before scheduling radical therapy**. The hybrid imaging method demonstrated a high detection rate for metastatic lesions – in 37.6% of the studied patients at intermediate and high risk. The detection of metastases from the primary PC is positively related to the EAU risk group, PSA and ISUP grade values. The detection rate of metastatic lesions is significantly higher in patients at high risk (42.6%) compared to intermediate-risk patients (6.6%). The detection rate for metastatic lesions: regional and distant LN, bone, visceral and distant metastases in total increases with increasing the PSA values, as well as the ISUP grade.
5. Possible diagnostic errors in the present study: false-positive and false-negative findings in PC staging were found in 13.8% and 7.3% of the examined patients, respectively. **Good knowledge of the different variations in physiological PSMA activity and possible causes for diagnostic errors is essential to optimise the interpretation of PSMA-PET results and for clinical practice, respectively, in determining the subsequent therapeutic approach.**

6. **The advantages of ^{68}Ga -PSMA PET/CT over CT in the initial staging of primary PC before radical therapy are mainly in detecting small size LN and bone marrow metastases** (PSMA PET found additional metastatic LN in 26.6% and bone metastases in 15.6% of patients). The detection of additional metastatic lesions by the hybrid method was positively correlated with the PSA levels.
7. **^{68}Ga -PSMA PET/CT is a highly promising hybrid imaging method exceeding conventional modalities in the detection of regional nodal and distant metastatic lesions in the initial staging of intermediate and high-risk primary PC** before a decision is made about radical therapy. PSMA PET/CT demonstrated a higher detection rate for regional nodal (42.0%) and distant metastatic lesions (44.9%), mainly in high-risk patients with higher PSA values ≥ 10.0 ng/mL and ISUP grade 4-5. Respectively, it introduces significant changes in the regional nodal (N) and distant (M) staging compared to the conventional imaging methods: in 30.4% of the N status patients and 37.7% of M status patients. Cases with N and M altered stages are more common in patients with a high-risk EAU group, and patients with Gleason scores 8-10. False-positive BSc results are high in 20.8% of BSc-positive patients (in terms of metastatic bone involvement).
8. **The present study results confirm the high potential of the hybrid imaging method PSMA PET/CT to replace both conventional modalities (BSc and CT) in the initial staging of high-risk patients.** Thus, we can optimise the diagnostic algorithm in primary PC and reduce the radiation exposure in these patients.
9. **^{68}Ga -PSMA PET/CT is a method with excellent potential and high frequency of PC detection when evaluating patients with ISUP grade 5:** for recurrent PC – 87.5% in patients with BCR after radical therapy, 77.3% in BP progression after RP, and for metastatic lesions in patients with primary PC – 73.9%. Malignant involvement of PC in patients with ISUP grade 5 is mainly due to distant metastasis (70.5% of patients). The detection rate is high for metastatic LN (regional and/or distant) (47.5%). The most common are external iliac and retroperitoneal (21.3%) lymph nodes. The detection rate for bone metastases is high (55.7%), with the most common cases for PSMA-active sclerotic bone lesions (34.4%). The incidence of false-negative bone findings is relatively high (11.5%). The increase in PSA values significantly increases the detection rate of distant LN, distant metastases in total, as well as the total detection rate (positive PSMA PET/CT results) in patients with BP progression after RP with ISUP grade 5. Statistically significant is the positive correlation between the detection rate for distant metastatic LN and the PSA values in patients with primary PC and ISUP grade 5. There is no significant correlation between the detection rate of malignant lesions and the clinical T stage.
10. **^{68}Ga -PSMA PET/CT is a highly promising imaging modality with excellent performance for detection of recurrent PC in BCR after radical therapy, biochemical progression after RP, and detecting metastatic lesions in primary PC staging, especially in well-differentiated PC.** In the present study we obtained high values for the parameters of the hybrid imaging method: detection rate, sensitivity (from 92.3% to 98.9%), specificity (97.7% – 100.0%), PPV (98.9% – 100.0%), NPV (69.2% – 97.7%) and accuracy (93.4% - 98.5%) in all groups of patients studied. Our patients' groups were: with BCR after radical therapy, with biochemical progression after RP (including very low PSA values < 0.2 ng/mL), patients referred for staging, and ISUP grade 5 patients. The detection rate is higher in patients with ISUP grade 5: for subgroup 1 (87.5%), subgroup 2 (77.3%),

subgroup 3 (73.9%) compared to the diagnostic I – III groups of patients. However, some other parameters of the method in patients with ISUP grade 5 – NPV (69.2%), sensitivity (92.3%) and accuracy (93.4%), have lower results compared to the first three groups: NPV (97.1% – 98.8 %), sensitivity (95.3% – 98.9%), accuracy (98.2% – 99.3%). The specificity and PPV in ISUP grade 5 are very high – 100.0%. Early detection of PC recurrence (especially important at low serum PSA levels), as well as the detection of metastatic lesions in PC staging (intermediate and high risk) with a diagnostic modality with high sensitivity, specificity, PPV, NPV and accuracy, such as the ⁶⁸Ga-PSMA PET/CT, is of critical clinical importance for the additional selection of patients in determining the subsequent therapeutic approach.

2. Recommendations for the clinical practice based on our experience

- 1. Following the principles of the individualised approach, the ⁶⁸Ga-PSMA PET/CT scan is justified in patients with biochemical progression after RP at low tumour marker values**, even with PSA < 0.2 ng/mL, given the established detection rate of recurrent PC in these patients (14.3% for PSA below 0.2 ng/mL) and a sensitivity (PSA-based) of 15.0% for PSA > 0.04 – 0.16 ng/mL).
- 2. The present study showcases the high potential of the PSMA PET/CT hybrid method to replace both conventional imaging modalities (BSc and CT) in the initial staging of high-risk PC patients**, which will therefore optimise the primary PC diagnostic algorithm and reduce the radiation exposure of these patients.
3. The present study results established a significantly higher detection rate of pathological PSMA-PET results in patients with ISUP grade 5 (group V) compared to the first three groups of patients (I, II, III), especially in terms of distant metastases detection. However, in the patients with ISUP grade 5, a relatively high frequency of false-negative findings (primarily bone) was also reported. Accordingly – lower NPV values were calculated compared to other diagnostic groups. Therefore, in order to exclude possible false-negative results, PSMA PET/CT scans in patients with ISUP grade 5 should be interpreted with caution, especially in patients with neuroendocrine differentiation of PC, as well as in restaging of patients with BCR with elevated PSA levels and a negative PSMA PET/CT scan. **In patients with ISUP grade 5, especially those with a confirmed lack of PSMA expression, following the principles of the individualised approach, an alternative imaging examination assessing high-risk PC should be considered – 18F-FDG PET/CT or ⁶⁸Ga-DOTA-TATE PET/CT in neuroendocrine differentiation of PC.**

VI. SCIENTIFIC AND APPLIED CONTRIBUTIONS

1. For the first time in Bulgaria's nuclear medicine practice, the innovative hybrid imaging method ^{68}Ga -PSMA PET/CT was applied in a large cohort of patients with BCR on PC after radical therapy (n = 133) investigated. The prognostic factors for the positivity of the PSMA-PET results were determined, as well as the factors related to the detection rate and the advantages of the method over CT imaging.
2. For the first time in the country, the application of the PSMA PET/CT method was studied in patients with biochemical progression after RP (n = 144) in a wide range of tumour marker values (with emphasis on low PSA levels, including < 0.2 ng/mL). The influence of PSA values on sensitivity and the frequency of PSMA-PET detection was analysed. The correlation between PSA values and the frequency of recurrent PC detection at different locations was determined. The correlation between the Gleason score and detection rate, the respective recurrence PC locations, performed ADT/hormone therapy, and PSA values were investigated.
3. For the first time in the nuclear medicine practice in Bulgaria, the application of PSMA-PET in the initial regional nodal (N) and distant metastatic (M) staging of patients with primary PC with intermediate and high risk (n = 109) before radical therapy was investigated. The advantages of PSMA-PET over conventional CT imaging have been determined.
4. An in-depth study of the application of the method in patients with ISUP grade 5 (n = 61) for the first time in the nuclear medicine practice in the country was performed. The specifics of nodal and bone metastasis, the correlation between detection rate for different localisations of malignant PC involvement and the PSA values, as well as the clinical T stage were analysed.
5. The role of ^{68}Ga -PSMA PET/CT in studying patients with intermediate and high-risk primary PC before radical therapy (n = 69) compared to conventional imaging methods (CT, MRI and BSc) for regional nodal (N) and distant metastasis (M status) assessment was emphasised. The factors related to the detection rate of regional LN and distant metastatic lesions were determined. For the first time in the nuclear medicine practice in Bulgaria, the impact of ^{68}Ga -PSMA PET/CT on staging (N, M) was analysed.
6. An in-depth study of the different anatomical models of metastatic involvement of recurrent and primary PC in ^{68}Ga -PSMA PET/CT scans (in a total of 386 patients) was performed.
7. The analysis of ^{68}Ga -PSMA PET/CT parameters (for the first time in the country) is of practical contribution: detection rate, sensitivity, specificity, PPV, NPV and accuracy, including the risk of false-positive and false-negative results in the different diagnostic groups of patients.
8. An in-depth study of the correlation between positive/pathological PSMA PET/CT results and the PSA values, Gleason score, ISUP grade, clinical T stage and other factors in patients with BCR after radical therapy (n = 133), with biochemical progression after RP (n = 144), as well as with primary PC (n = 109) in a total of 386 patients was performed.
9. To optimise the interpretation of PSMA-PET results, an in-depth analysis of possible diagnostic errors was performed, including variations in the physiological PSMA activity, the pathological expression of non-PC-related PSMA antigen, and the false-negative findings.

10. The recommendations for applying the hybrid imaging method in patients with biochemical progression after RP (low PSA values), in the initial staging of high-risk PC, and patients with ISUP 5 are of practical importance.

VII. PUBLICATIONS AND PARTICIPATION IN SCIENTIFIC FORUMS RELATED TO THE DISSERTATION

1. PUBLICATIONS

Dyankova M. Possible diagnostic Pitfalls in ^{68}Ga - PSMA PET/CT Interpretation. Varna Medical Forum, Suppl. 1. Proceedings from IX Scientific Session of Medical College of Varna, 2021, 10 (1): 42-49.

2. INTERNATIONAL CONFERENCES AND CONGRESSES

Dyankova M., Dancheva Z., Stoeva. T., Chausheva S., Yordanova T., Chaushev B., Klisarova A. Diagnostic value of Ga- 68 PSMA PET/CT in biochemical progression in prostate cancer patients after radical prostatectomy in the low range of prostate-specific membrane antigen (PSA). Annual Congress of the European Association of Nuclear Medicine, October 22 – 30. EANM'2020. Eur J Nucl Med Mol Imaging (2020) 47 (Suppl 1): p. 365.

3. NATIONAL CONFERENCES AND CONGRESSES

Dyankova M. Possible diagnostic errors in the interpretation of ^{68}Ga -PSMA PET/CT. IX Scientific Session for Professors and Students of the Medical College. Varna, March 26, 2021

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