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Abbreviations: AC1, Gwet's first-order coefficient; IQR, interquartile range; MIBI-kidney, ^{99m}Tc-sestamibi combined with single-photon emission computed tomography/computed tomography of the kidney.

The oncological characteristics of non-prostate-specific membrane antigen (PSMA)-expressing primary prostate cancer on preoperative PSMA positron emission tomography/computed tomography

Approximately 5% of patients with D'Amico intermediate- to high-risk, hormone-sensitive prostate cancer (PCa) have a tumour that lacks prostate-specific membrane antigen (PSMA) expression on preoperative positron emission tomography (PET)/CT (non-PSMA$_{PET}$-expressing PCa) [1]. Our recent study showed that patients with non-PSMA$_{PET}$-expressing PCa had similar clinical, pathological, and immunohistochemical characteristics to patients with PSMA$_{PET}$-expressing tumours, suggesting that these patients may have similar prognostic features [1]. In the present study, oncological outcomes (i.e., biochemical recurrence [BCR] and detection of recurrent disease on restaging PSMA PET/CT in case of BCR) of patients with non-PSMA$_{PET}$-expressing PCa were studied.

This study was approved by the local Institutional Review Board (IRBdm19-348). A retrospective, cross-sectional cohort was created consisting of all patients who had hormone-sensitive, D'Amico intermediate- to high-risk PCa, who underwent a preoperative PSMA PET/CT, and who underwent robot-assisted radical prostatectomy (RARP) in a tertiary referral centre between 2016 and March 2020 [1]. The cohort was extended with five additional patients, collected during multidisciplinary oncological board meetings, with non-PSMA$_{PET}$-expressing PCa who underwent RARP between March 2020 and September 2021. Patients underwent PSMA PET/CT with different radiotracers: ^{68}Ga-PSMA-11, ^{18}F-DCFPyL, ^{18}F-PSMA-1007, or ^{18}F-JK-PSMA-7. We did not perform subgroup analyses per radiotracer, because this would lower the power of the analyses and the tracers have similar staging accuracy [2]. All preoperative PSMA PET/CT scans were centrally reviewed by experienced nuclear medicine specialists. Non-PSMA$_{PET}$-expressing PCa was defined as no focal tracer uptake in the prostate visually exceeding the background activity of normal prostate tissue, in accordance with Prostate Cancer Molecular Imaging Standardised Evaluation (PROMISE) criteria and the European Association of Nuclear Medicine standardised reporting guidelines for PSMA-PET (E-PSMA) [3,4].

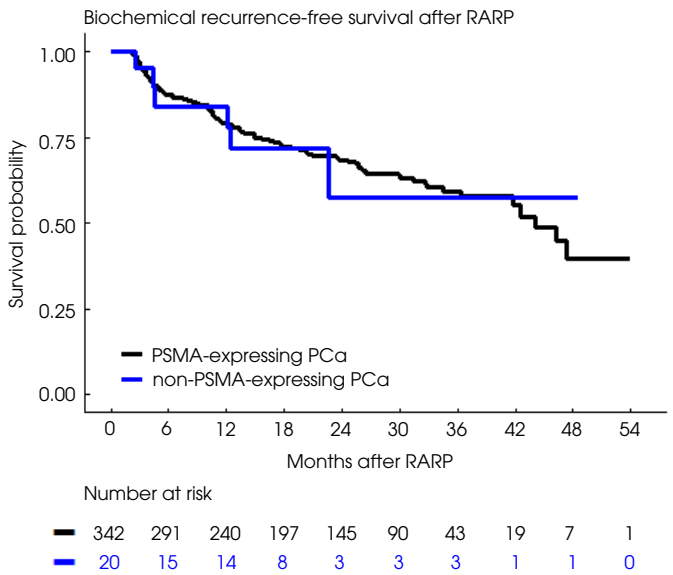
After RARP, patients received oncological follow-up at 4, 8, 12, 18, 24 months after surgery and yearly thereafter. At each visit, the PSA level was measured. We excluded five patients due to missing PSA data. In case of BCR (i.e., PSA level ≥0.2 ng/mL), a restaging PSMA PET/CT imaging was performed according to European Association of Urology (EAU) guidelines recommendations and interpreted according to local expertise. Pathological data, PSA values, tracer type and clinical reports of restaging PSMA PET/CT were collected.

Differences between patients with non-PSMA$_{PET}$-expressing and PSMA$_{PET}$-expressing PCa were analysed. Categorical variables were analysed with the Fisher's exact test.

Differences regarding BCR-free survival were analysed with multivariable Cox regression tests using known prognostic factors as covariates (pT3 stage, positive surgical margin, pathological Gleason score, and pathological nodal stage). Patients were censored at the time of last PSA test. Statistical significance was set at $P \leq 0.05$.

A total of 20 patients had non-PSMA$_{PET}$-expressing PCa and 342 had PSMA$_{PET}$-expressing PCa. No differences in pathological characteristics (pT3 stage, Gleason score, positive surgical margin rate or pathological nodal stage) were found between the two cohorts (Table S1). The median follow-up time for those who did not develop BCR was 25 (interquartile range [IQR] 16–32) months. BCR developed in six patients with non-PSMA$_{PET}$-expressing PCa and in 117 patients with PSMA$_{PET}$-expressing PCa. Restaging PSMA PET/CT was performed in 116/123 (94%) patients who developed BCR. The median PSA at the time of restaging PSMA PET/CT was 0.31 (IQR 0.22–0.61) ng/mL, with similar distribution between the cohorts ($P = 0.34$, Table S1). There were no differences in BCR-free survival (adjusted hazard ratio [HR] 1.3, 95% CI 0.6–2.9; $P = 0.58$) between the cohorts using the larger cohort (PSMA$_{PET}$-expressing PCa) as reference (Table S2, Fig. 1). The estimated 12-month BCR-free survival rate was 84% (95% CI 69%–100%) for patients with non-PSMA$_{PET}$-expressing PCa and 79% (95% CI 74%–83%) for patients with PSMA$_{PET}$-expressing PCa. Two patients with a preoperative non-PSMA$_{PET}$-expressing tumour had PSMA$_{PET}$-expressing bone metastases on restaging PSMA PET/CT (Fig. S1). One patient with a preoperative non-PSMA$_{PET}$-expressing tumour had a negative restaging PSMA PET/CT, but an adenocarcinoma-positive biopsy of a prominent lymph node (short axis diameter 8 mm) (Fig. S2). Immunohistochemical staining of the lymph node biopsy with anti-PSMA mouse antibody (Clone 3E6; DAKO, North America Inc., Carpinteria, CA, USA) at a 1:100 dilution confirmed the presence of intense membranous PSMA protein expression.

Fig. 1 Kaplan–Meier curves with separate lines for patients with non-PSMA$_{PET}$-expressing and PSMA$_{PET}$-expressing PCa showing BCR-free survival.



This is the first study to report on the oncological outcomes of patients with non-PSMA$_{PET}$-expressing PCa. This study shows that patients with non-PSMA$_{PET}$-expressing PCa have similar BCR-free survival rates to patients with PSMA$_{PET}$-expressing PCa. This corroborates with our previous observation that these patient groups have similar prognostic features [1]. The results contradict the theory that non-PSMA$_{PET}$-expressing tumours are associated with neuroendocrine differentiation and worse prognosis [5]. Therefore, patients with non-PSMA$_{PET}$-expressing PCa should be offered the same treatment options as patients with PSMA$_{PET}$-expressing PCa.

An important finding is that two patients with initial non-PSMA$_{PET}$-expressing PCa developed bone metastases with PSMA expression on restaging PET/CT. These metastases were not confirmed through biopsy, but the PSA level decreased after metastasis-directed radiotherapy, indirectly confirming the presence of metastatic disease. Therefore, PSMA PET/CT seems a valuable tool for restaging PCa, even in patients with BCR who had a non-PSMA$_{PET}$-expressing PCa on preoperative scans. To the best of our knowledge, this finding has not been reported previously. The PSMA expression of the bone metastases may have clinical implications as PSMA-targeted radioligand therapy (i.e., ^{177}Lu-PSMA-617) may be a viable option for initially non-PSMA$_{PET}$-expressing PCa, even though this therapy is, at present, reserved for patients who are in more advanced stages of the disease.

False-negative findings on molecular imaging (similar to non-PSMA$_{PET}$-expressing PCa) occur in other tumour types as well. Potential causes of false negativity in the molecular imaging of, e.g., neuroendocrine tumours (NETs), are small volume disease (<5 mm), disease masked by normal physiological activity in the surrounding tissue, atypical subtype expression, or poorly differentiated phenotype of the tumour [6]. Unlike molecular imaging in NETs, false-negative findings in PCa are not caused by small volume or poorly differentiated phenotype, as reported in our previous study [1]. The four different splice variants of PSMA (PSM', PSM-C, PSM-D, and PSM-E) may affect the progression and development of PCa, but little is known about their protein function [7]. It is possible that the splice variants have lower

affinity for PSMA radiotracers, which may result in the absence of PSMA expression on PET/CT. Other (partial) contributions to the phenomenon of non-$PSMA_{PET}$-expression may be technical factors with respect to PSMA PET/CT imaging itself or due to the biodistribution of the radiotracer.

The small group size of the patients with non-$PSMA_{PET}$-expressing PCa limits the statistical power of the analyses. Some patients underwent preoperative PSMA PET/CT in a referring centre with potentially different PET protocols. This heterogeneity may lead to inaccuracies of the radiological staging. Nonetheless, our research group recently showed that the referring and tertiary referral centre nuclear medicine specialists had an excellent inter-observer agreement in tumour staging [8]. We may have introduced bias by adding the five patients who were operated at a later time to the non-$PSMA_{PET}$-expressing group. Due to the shorter follow-up, we may have missed the BCRs developed at later time points. The median (IQR) PSA follow-up was longer for $PSMA_{PET}$-expressing PCa, at 27 (21–35) vs 21 (14–34) months. Therefore, we did not report the *P* values regarding the BCR-free survival analyses but only reported CIs.

In conclusion, after RARP, patients with a 'negative-for-PCa' preoperative PSMA PET/CT had similar BCR-free survival to patients with $PSMA_{PET}$-expressing PCa. A restaging PSMA PET/CT seems to be valuable in the detection of recurrences in patients with non-$PSMA_{PET}$-expressing PCa who developed BCR.

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Disclosure of Interests

The authors have no conflicts of interest to report.

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Abbreviations: BCR, biochemical recurrence; HR, hazard ratio; IQR, interquartile range; NET, neuroendocrine tumour; PET, positron emission tomography; PSMA, prostate-specific membrane antigen; RARP, robot-assisted radical prostatectomy.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Pathological and oncological outcomes and radiological findings.

Table S2 Cox regression analysis for biochemical recurrence-free survival.

Fig. S1 Two patients with initially non-$PSMA_{PET}$-expressing PCa had $PSMA_{PET}$-expressing bone metastases on restaging PSMA PET/CT after biochemical recurrence.

Fig. S2 A patient with a pT3bN0 R1 Gleason score 4 + 5 prostate cancer had biochemical progression after RARP.

